A STUDY OF YEASTS OF MARINE ORIGIN

SUMMARY

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The thesis is introduced by a review of the literature concerning the occurrence and composition of the yeast flora of marine fish and other parts of the marine environment.

The experimental work can be divided into three sections:-

The first of these sections concerns the collection and maintenance of yeast cultures from various marine sources. Altogether 235 such cultures were obtained and of these, during this survey, 189 were isolated from marine fish and 11 from sea-water. The remaining 35 were isolated by other workers. Improved methods for sampling and subsequent isolation of yeasts are described.

The second experimental section deals with the identification of these isolates using the system of Lodder and Kreger-van Rij (1952). The 213 isolates from marine fish were found to comprise eight genera - Debaryomyces, Torulopsis, Candida, Rhodotorula, Pichia, Trichosporon, Cryptococcus and Pullularia. D.kloeckeri comprised 45% of these isolates and T.inconspicua(var) and C.parapsilosis each 11% respectively.

The 22 isolates from other marine sources belonged to the same genera as the fish isolates and in addition five of the eleven strains isolated from sea-water samples in this survey were classified as Metschnikowia krissii (van Uden and Castelo-branco)nov.comb.

Although most of the isolates closely resembled the descriptions of the type species some differences were obvious and these have been discussed in the light of possible strain variation within the species.

The third section of experimental work comprises further studies of the isolates. The results obtained from these studies and their probable value in establishing strain variation have been discussed.

Infra-red spectrophotometry of whole cells, soluble and insoluble cellular material was found to be of little value for species or strain differentiation. It was conjectured that identical cellular components producing specific spectra occur in the different species and mask the effects of differences in other cellular components.

Qualitative studies of the free amino-acid pools of certain of the marine isolates and terrestrial strains of corresponding species also indicated that strain characterisation on this basis is impracticable. No outstanding qualitative differences between the compositions of the free amino-acid pools of the marine and terrestrial strains were apparent.

The maximum concentration of NaCl tolerated by the marine isolates was found to be a feature of each strain and there was a correlation regarding halo-tolerance among strains of a particular species isolated from different marine locations. Complex organic nitrogen sources were found to stimulate halo-tolerance in the isolates. As the concentration of NaCl was increased in the growth medium a prolongation in the yeasts' lag phase was observed but their growth rate remained fairly constant.

Strains/

Strains of D.kloeckeri were found to exhibit optimal growth in media containing 1.0 to 3.0% NaCl, whereas the other species tested grew best without NaCl.

The thesis concludes with a discussion concerning the occurrence and species distribution of the yeasts isolated during this survey.

Skin samples had the highest incidence of yeast occurrence; the samples from the gills and mouth exhibited the next highest incidence, while a much lower value was recorded in those from the faeces.

Strains of D.kloeckeri were predominant among the isolates from every location of sampling, whereas the occurrence of other species and the proportion of isolates comprising them varied in the different geographical areas. Results also indicate that different fish species may carry their own characteristic yeast flora.

A T H E S I S

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C O N T E N T S

P R E F A C E

The bulk of marine biological research to date has been concerned with the utilisation of marine biota as food resources for man. Marine microbiology has made some important contributions in this field, especially with regard to micro-organisms pathogenic for marine animals and those causing spoilage of marine materials. Only a small proportion of the micro-organisms comprising these two categories, however, was found to be yeasts and therefore these organisms have received but scant attention from marine microbiologists in past years.

It is hoped, therefore, that this study of yeasts collected from the marine environment, mainly from fish, may be a useful contribution to the rapidly expanding field of marine microbiology.

INTRODUCTION AND REVIEW

In the last sixty years many reports have appeared regarding the large bacterial population associated with marine fish, the earlier of which included those by Forster (1887), Reed and Spence (1929), Sanborn (1930), Stewart (1932), Gibbons (1934), Wood (1940) and recently these bacteriological investigations, especially of commercially important species of fish, have been intensified, e.g., Dyer (1947), Shewan (1949), Reay and Shewan (1949), Liston (1955, 1956, 1957), Georgala (1957, 1958). However, despite this increasing interest in the nature of the micro-flora of marine fish, at the time of undertaking this study scant information existed regarding yeasts as a specific part of this micro-flora. Most of the reports of the occurrence of the latter micro-organisms in this association have been incidental to primary bacteriological investigations and fail to describe the organisms in detail, thus making identification uncertain or impossible. The following reports are typical examples of such:-

During an investigation of the bacterial flora of salmon in North Pacific waters, Snow and Beard (1939) found that yeasts comprised from one-half to one per cent., of the micro-organisms isolated from samples from gills, skin and anus. No descriptions of the yeasts were, however, provided by these workers. Wood (1940) isolated sixteen strains of yeasts from various marine sources in Eastern Australian waters. Thirteen of these strains, which were described/

described as "pink and very poorly reactive", included five from mullet slime and three from flathead slime. One "white yeast" was also isolated from mullet slime and two "black yeasts" from flathead gills; again no further identification of the isolates was made. Also from the skin of a live shark caught in waters off New South Wales, Australia, the latter worker, Wood (1950), isolated a yeast which he described as a "pink torula" and in the course of bacteriological investigations in the same waters (1953) he isolated a further thirty-two yeasts of which twenty-seven are described as "pink and unreactive", three as "white yeasts" and two as "black yeasts". Thirteen of these isolates were from teleosts, one from an elasmobranch and the remainder from sea-water and mud-samples taken in the same area as the fish. It is probable that the yeasts described as "pink and unreactive" and as "pink torula" were strains of Rhodotorula and that "black yeasts" belonged to the genus Pullularia. The description of "white yeasts", however, is too vague to permit even a provisional diagnosis.

Dyer (1947) while investigating the micro-flora of Atlantic cod (Gadus callarias), obtained thirty-eight isolates which he described as "yeast-like" organisms. Thirteen of these were found in slime from live fish, ten in fish faeces and twelve were isolated from fish stored for some time on board the fishing vessel. A group of thirty-one of these isolates is described as being "probably rose-coloured torula" (probably species of Rhodotorula); another group comprised six "white torula" and one "dark brown yeast producing/

producing four spores". No more details of these yeasts' characteristics were reported, thus making their further identification impossible. These"yeast-like organisms", together with some "mould-like organisms", comprised 1.0% of the total isolates from samples incubated at 20° C., and this figure increased to 13.3% in the case of the samples held at 4° C.

During an investigation of the bacterial flora of skate (Raja spp.) and lemon sole (Pleuronectes microcephalus) in the North Sea, Liston (1957) found 4% of the micro-organisms isolated at 37° C., from skate were yeasts and moulds; under similar conditions no yeasts or moulds were detected in the lemon sole. Liston, however, carried out most of his isolations at 20° C., and 0° C., but has made no mention of finding yeasts at these temperatures. Also on record is the isolation by Konokotina and Krassilnikow (1930) of a yeast strain described as resembling Debaryomyces tyrocola (now designated Debaryomyces hansenii by Lodder and Kreger-van Rij, 1952) from the intestine of a cetaceous mammal, the porpoise.

Several other reports have also been made of the occurrence of yeasts on marine animals, apart from fish. Hunter (1920) found that a "pink yeast" was responsible for the spoilage of oysters during shipment from New England beds. This organism, termed "torula", which grew readily at low temperatures, i.e., 6° C., was also found to be present in the surface as well as the bottom water of the oyster-beds and on 26.3% of healthy oysters sampled. From frozen oysters McCormack (1950) also isolated "pink yeasts" which could grow at temperatures/

temperatures ranging from 0° F., to -30° F., and reproduced on oysters held at 0° F. In a further examination of these yeasts McCormack (1956) claims to have observed asci with one to four spores, a fact which makes identification of these yeasts extremely difficult unless they were possibly Sporobolomyces.

The occurrence of yeasts on shrimps (Peneaus setiferus) has been reported by Phaff et al. (1952) who isolated thirty-five yeast strains in the course of a three-year bacteriological study of these animals in the Gulf of Mexico. In contrast with the foregoing reports, the isolated strains were investigated fully for taxonomic characteristics, salt tolerance and temperature range over which growth occurred. Eleven strains were classified as Rhodotorula, spp., nine as Trichosporon spp., three as Torulopsis spp., four as Pullularia spp., six as Candida spp., and two as Hansenula spp. Amongst these they described five new species - Trichosporon diddensii, Tr.lodderi, Rhodotorula peneaus, Rh. texensis, Rh.marina - and a new variety Trichosporon cutaneum var. peneaus.

Microbiological studies of edible marine shell-fish from Japanese waters were undertaken by Kobayasi et al. (1953) and Nakazima (1954, 1955, 1957) who observed yeasts in the gut and mid-gut gland of the little-neck clam (Venerupis semidecussata) and the oyster (Ostria gigas). From the mid-gut gland of the former animals the following yeast species were isolated - Candida humicola, C. pelliculosa, Candida/

Candida spp., Debaryomyces kloeckeri, Hansenula anomala, Pullularia pullulans (Berkhout), Rhodotorula minuta, Rh. mucilaginosa, Rh. rubra and Torulopsis aeria.

Since this present survey was started the results of several studies dealing specifically with the yeast micro-flora of various marine materials, including marine animals, have been published. Fell and Roth (1961) in an extensive investigation of the ecology and taxonomy of yeasts occurring in waters near South Florida and The Bahamas isolated one hundred and ninety-one yeast strains from algae, grasses, sea-water, sediments and faecal contents of fish collected in these areas. Approximately 70% of the fish of diverse genera (unspecified) proved to have yeasts in their faecal contents among which species of the genera Candida, Rhodotorula and Trichosporon predominated. The qualatitive and quantitative data collected during this investigation is not given in the above report but it appears that the authors are suggesting that in the marine environment the intestinal contents of fish do not appear to be an important site of yeast propagation when compared with off-shore waters and sediments where abundant yeast populations were detected.

Roth et al. (1962) reported further data resulting from a continuation of the above investigation, in which one hundred and twenty-seven yeast strains, comprising sixteen species, were isolated from the intestinal tracts of 61% of the marine teleosts examined.

From/

From the majority of fish only one or two yeast species were obtained per specimen. In fish caught in inshore waters, in Biscayne Bay, Florida, Trichosporon cutaneum was the most commonly occurring yeast species, being found in 50% of the fish sampled. Strains of the species Candida parapsilosis, C. tropicalis, C. guilliermondii, Rhodotorula rubra, Rh. pilimanae, Hansenula anomala, Debaryomyces kloeckeri, Hanseniaspora valbyensis were also isolated. The fish catch comprised twenty-two specimens Haemulon spp., one Carena spp., and one Anisotremus spp.

In December, 1960 and in January, 1961 the intestinal contents of fish caught near the tropical island of Bimini, The Bahamas, were sampled. From the first catch Rhodotorula minuta was found to be the most prevalent yeast species, being isolated from fourteen of the thirty-one fish sampled. Also isolated in this instance were strains of Rhodotorula glutinis and Candida parapsilosis. Strains of the latter species were found to predominate amongst the yeast isolates from the second fish catch from which strains of Candida tropicalis Rhodotorula pilimanae and Torulopsis spp., were also isolated. Fish comprising the first catch were fifteen specimens Haemulon spp., ten Stenotomus spp., four Ocyurus spp., one Anisotremus spp., and one Lachnolaimus spp., whereas the second catch comprised two Seriola spp., two Balistes spp., one Melacanthus spp., one Halichoeres spp., two Carena spp., One Halecentrus spp., one Anisotremus spp., and two unidentified specimens.

Yeasts were also isolated during this study from the gut contents of/

of crustaceans of which one hundred and thirty-seven samples representing ten genera yielded yeasts in nineteen (14%). From the whole washed bodies or gut contents of lower invertebrates e.g., copepods, higher numbers of yeasts were isolated. Eight of the fourteen samples (57%) representing eight genera of these animals yielded yeasts. The surface washings from the last two groups of animals were also found to contain yeasts and this flora generally resembled more closely that of the adjacent waters and sediments than that from the animals' gut contents or whole washed bodies.

Regarding the occurrence of yeasts in the intestinal contents of fish sampled during these studies Roth et al.(1962) reached a conclusion similar to that of Fell and Roth (1961), i.e., that "in this location yeast species appear to be of transitory nature and restricted in population density." As no figures of yeast population densities in fish have been published by the latter workers it must be inferred that they have drawn their conclusions from the small number of different yeast species obtained from each fish, and also the fact that yeasts were found in only 61% of the fish samples examined.

In the course of an investigation of the yeast flora of various marine materials from the same area in which the latter studies were made, Capriotti (1962)[c] also examined the intestinal contents of seventeen species of marine animals, mainly fish, and yeasts were found in only ten of the fifty-seven samples(20%). The most prevalent yeast strain described as Candida I spp. was found in four fish/

fish and also isolated were strains of Cryptococcus albidus, Debaryomyces subglobosus and Aureobasidium pullulans.

Two new yeast species - Metschnikowia krissii and M. zobellii - isolated in the Pacific Ocean have been described by van-Uden and Castelo-branco (1961). Both species were found in sea-water but the latter was also found in greater numbers in the gut contents of topsmelt (Anthenrinopis affinis littoralis) and Pacific Jack mackerel (Trachurus symmetricus) which were caught off the coast of La Jolla, California. This genus was first described by Metschnikoff (1884) who found these yeast-like organisms in the body cavity of Daphnia magna, a fresh-water crustacean. Both new species were found capable of parasitising this animal under experimental conditions and the authors suggest that they may also be capable of facultatively parasitising marine crustaceans and other organisms on which fish feed and thus gain entry to the fish gut. In this latter connection Kriss and Novozhilova (1954) have some evidence that yeasts may play an important part in the nutrition of the marine worm, Nereis succinea, which forms the bulk of the zooplankton in the Sea of Azov and Caspian Sea and which may well serve as a food source for many fish. Another new yeast species of marine origin, Torulopsis haemulonii, has recently been described by van-Uden and Kolipinski (1962). Strains of this species were isolated from the gut contents of a fish, Haemulon sciurus, Shaw ("bluestriped grunt") caught off Miami, Florida and also from a water sample from Biscayne Bay and from sea-water collected in the Atlantic Ocean, off the/

the coast of Lisbon, Portugal.

Another recent contribution concerning yeasts from marine habitats is a paper by Siepmann and Höhnk (1962), describing the isolation and identification of yeasts and fungi from samples of various marine materials, e.g., fish shrimp eggs, sea-cucumbers, etc., taken during two voyages in the North Atlantic Ocean. Altogether one hundred and twenty yeast cultures were obtained in this study of which fifty-one were obtained from fish and comprised Debaryomyces subglobosus (20), Trichosporon cutaneum (13), Hensensula californica (6), Rhodotorula glutinis (4), Debaryomyces kloeckeri (2), Rhodotorula mucilaginosa (2), Cryptococcus albidus (1), Torulopsis candida (1), Pullularia pullulans (1) and a new species, Trichosporon piscium (1). From other marine sources cultures were obtained of Cryptococcus laurentii, Rhodotorula rubra, Rh. texensis (Phaff et al., 1952) and two new species Trichosporon atlanticum and Trichosporon maritinum.

In a recent bacteriological study of distant water cod landed at Port of Hull, Spencer (1961) reported finding yeasts in a number of samples incubated at 20° C. From Bear Island cod, yeasts comprised 3% of the isolates from one hundred and sixteen skin samples and 4% of the twenty-three flesh samples, while from cod caught off the Norwegian coast yeasts comprised 2% of the isolates from both the hundred and one skin samples and the hundred and thirty-nine flesh samples. No further description of these isolates was made. De Silva (1960) also reported that a number of yeasts - yellow, red, pink and white - with a variety of morphological features, were isolated with relative ease during a bacteriological study of North Sea herring although/

although selective media for yeasts were not employed.

The results of many bacteriological studies have indicated that there is a general similarity between the bacterial flora of fish and their surrounding marine environment - Zobell (1946). Further evidence that this environment may be the source of many organisms found on fish is the discovery of some correlation between plankton outbursts, which must initially affect the sea-water micro-flora, and seasonal increases in the number of bacteria found on fish - Liston (1956), Georgala (1958). As yet little data is available to indicate whether the yeast micro-flora of fish is similarly influenced, but in view of these findings regarding bacteria it may be profitable also to consider reports of yeast isolations from marine sources, other than fish, such as sea-water, bottom silts and muds, marine plants and other marine animals. Reports regarding yeast occurrence in the last-named location, i.e., other marine animals, have, for reasons of convenience, been already discussed and it is now proposed to review reports of yeasts found in other parts of the marine environment.

The earliest reports of yeasts in the seas were those by Fischer (1894) who indicated their wide distribution in the surface layers of the Atlantic, since they were not only found in coastal waters but also at a distance of several hundred miles from land. Furthermore, this worker claimed that the frequency of yeast occurrences appeared greater in the higher latitudes and that no yeasts were found below about/

about 31°. The isolates were described as "white and red torula species", non-fermentative and non-film-forming. Also recorded were "black yeasts", a mycoderma-like form and two strains isolated in the Skagerrak described as resembling Saccharomyces ellipsoideus II and S.pastorianus II. A yeast strain, found near the Azores Islands, was described by Fischer and Brebeck (1894) in detail and called Blastoderma salmonicolor. This species was later designated Sporobolomyces salmonicolor by Lodder and Kreger-van Rij (1952).

Issachenko (1914) while investigating the bacterial flora of the Arctic Ocean reported the isolation of rose-coloured and black yeast strains. The former strains were described briefly with regard to their morphological and cultural properties but were not identified and the two "black yeasts" were described as a new species - Nadsonia nigra (Issachenko,1929). From the surface of the various species of marine algae collected on the Murmansk coast of the same ocean, Nadson and Burgvitz(1931) isolated twenty-two yeast strains, fifteen of which were described as "white torula species" and seven as "red torula species". About half of the "white torula species" could not ferment carbohydrates or did so only slightly, the remainder being able to ferment a number of carbohydrates; all these yeasts grew well at 2°C to 4°C. Kudriavtzev (1932, 1933) also reported the isolations of yeasts from the surface of marine algae which was collected on the far-eastern shores of the Pacific. In his collection of forty-three strains one was described as a new species - Nadsoniomyces sphenoides.

As/

As was the case with the reports concerning yeasts occurring on fish, the early authors of the above studies gave scant descriptions of the isolated organisms, thereby making their identification impossible in terms of present-day nomenclature. Nevertheless, their reports serve as an indication of the widespread occurrence of yeasts in the world's seas and oceans. The reports of Zobell and Feltham (1934) and Zobell (1946) of the occurrence of yeast colonies on most culture plates inoculated with marine materials collected in the open waters of the Pacific, as well as in water near the Californian Coast, also substantiated the fact that yeasts are ubiquitous members of the marine micro-flora.

In recent years more detailed studies dealing specifically with this marine yeast micro-flora have been undertaken. A generous contribution to this ecology has been made by contemporary Soviet microbiologists, a team of whom began extensive investigations of the micro-flora of the Black Sea in 1946 - Kriss (1959). During the years 1946, 1948 and 1949, water samples were taken from various depths, i.e., from surface to bottom, at stations situated not only in coastal regions but up to sixty miles off-shore in deep waters, i.e., 2,000 metres. During these years only five yeasts were isolated from two hundred samples and from these observations it was concluded that the Black Sea is a poor milieu for yeast life. However, in 1949 the then new method involving the use of membrane filters facilitated the sampling of larger volumes of water. This technique then revealed budding yeast cells, even in waters from the greatest depth. This sampling technique

was/

was thus employed for further sampling during 1950 by Kriss, Rukina and Tikhonenko (1952) and achromogenic , chromogenic and fungal-like forms of yeasts were found at most depths in small numbers, i.e., less than one per ml. Forty cultures obtained during this investi-:gation were classified according to Lodder and Kreger-van Rij (1952) and Kudriavtzev (1954) and a fuller description of them was given by Rukina and Novozhilova (1952). They were placed in three families, i.e., Rhodotorulaceae (Lodder), Torulopsidaceae (Lodder) and Saccharomycodaceae (Kudriavtzev), as in Table 1.

TABLE 1

Species composition of yeasts isolated from various parts of the Black Sea - Rukina and Novozhilova (1952):-

Rhodotorulaceae	Rhodotorula glutinis var. infirmo-miniata
	Rhodotorula rufula
	Rhodotorula mucilaginosa var. sanguinea
Torulopsidaceae	Torulopsis pulcherrima
	Torulopsis minor
	Torulopsis candida
	Torulopsis laurentii
	Torulopsis aeria
Saccharomycodaceae	Hanseniaspora apiculata

Also isolated, but not indicated in Table 1, were yeast-like organisms with well defined mycelia resembling Myco-toruloideae. During this survey the most widely occurring yeast species in the Black Sea were found to be Torulopsis pulcherrima, T.minor, T.laurentii and Rhodotorula rufula. It is surprising to note, in view of the findings to the contrary in this present survey and the reports/

reports of other workers, that sporing yeasts were not found in these waters even in samples from shallow, littoral areas.

Between 1950 and 1953 these microbiological investigations continued in the Black Sea and were also extended to the deep water areas of the Sea of Okhotsk and adjoining regions of the Pacific Ocean - Kriss and Novozhilova(1954), Novozhilova (1955), Kriss (1959). The majority of the sampling stations were placed over open waters up to one hundred and forty miles from the nearest land. Such stations allowed samples to be taken from the surface layers to depths as great as 4,800 metres. At a few stations seaweed catches and plankton from various strata ranging from the surface to 2,000 metres were also investigated.

Results of the studies revealed the widespread occurrence of yeasts throughout these areas, although the numbers occurring in superficial strata and shallow littoral zones were generally proportionately greater than in pelagic waters or the open seas, respectively. The richest source of yeast, however, proved to be the plankton catches of which 90% of those in the Black Sea and 50% of those in the Sea of Okhotsk and the Pacific were found to contain yeasts, the numbers of which ranged from 2,000 cells/litre to 36,000 cells/litre. Only one in ten of the samples of the surface layers of mud from the deep parts of the Sea of Okhotsk and the Pacific Ocean was found to contain yeasts.

On some occasions quite high numbers of yeast cells were detected/

detected in samples taken from considerable depths, e.g., at 2,500 metres in the Okhotsk-Pacific region the number of yeasts, as determined both by direct count and plate count, was 2,583 cells/litre of seawater and 4,710 colonies/litre of sea-water, respectively. Finds of this nature suggested to Novozhilova (1955) that yeasts in these areas may exist in "zones" and identification of the isolates did indicate definite "zoning" of certain yeast species. As well as this "zoning" some stratification of yeast species was obvious from the results, i.e., occurrence of the same yeast species at the same depths in widely separated localities. As yet little is known of the stability of these "yeast zones and strata", but if some hydrographic data (temperature, salinity, etc.) had been included in the above reports their existence could perhaps have been adequately explained.

This finding of viable yeast cells in considerable numbers at great depths far from the coast is of tremendous interest because it indicates that the particular conditions existing for life in these locations, i.e., low temperatures, high pressures, etc., do not preclude the occurrence of yeasts. The detection of the organisms well below the region of zoo- and phyto-plankton, i.e., below 2,000 metres, suggested to the investigators that the organisms are capable of utilising directly the available organic material in sea-water and results of experiments carried out by these workers in which yeasts were found capable of growth in unsupplemented sea-water/

sea-water seem to substantiate this hypothesis. The ability of the yeasts to cause denitrification was also suggested as a means whereby the organisms obtain oxygen in regions where it is in short supply but where inorganic nitrogenous substrates are available, such as in certain parts of the Black Sea.

Some five hundred and twenty-five cultures obtained during these latter studies were classified into four families, namely, Torulopsidaceae (Lodder), Rhodotorulaceae (Lodder), Sporobolomycetaceae (Lodder) and Saccharomycodaceae (Kudriavzev), as indicated in Table 2.

TABLE 2

Species composition of yeasts isolated from the Black Sea, Sea of Okhotsk and Pacific Ocean - Novozhilova (1955):-

Torulopsidaceae	Torulopsis pulcherrima	Torulopsis lipofera
	Torulopsis candida	Torulopsis laurentii
	Torulopsis aeria	Torulopsis neoformans
	Torulopsis minor	
Rhodotorulaceae	Rhodotorula glutinis var. infirmo-miniata	
	Rhodotorula glutinis var. rubescens	
	Rhodotorula mucilaginosa	
	Rhodotorula pallida	
	Rhodotorula colostri	
	Rhodotorula aurea	
Sporobolomycetaceae	Sporobolomyces salmonicolor	
Saccharomycodaceae	Hanseniaspora apiculata.	

Also isolated was a strain resembling Rhodotorula rufula, one resembling Rh. sanniei and small yeast-like, asporogenous organisms with well defined mycelia.

It can be seen that although a wider range of species was

obtained/

obtained in this study, the general pattern is similar to that described by Rukina and Novozhilova (1952) in their studies confined to the Black Sea. Many of the species listed in Table 2 possessed different strains, e.g. Rhodotorula glutinis, var. infirmo-miniata, Torulopsis pulcherrima were represented by fifteen and eleven different strains, respectively. These strains were differentiated by the authors by small variations in morphological features and also by certain differences in their physiological properties, e.g., ability to liquify gelatin, ability to utilise certain nitrogen-containing compounds as their sole source of nitrogen, etc. Altogether some eighty-five strains were described amongst the sixteen species in the collection. Sporobolomyces salmonicolor, strain A was found to have the most widespread occurrence in the locations sampled and Rhodotorula aurea, Rh. mucilaginosa, Rh. pallida, Rh. glutinis, var. infirmo-miniata and Torulopsis candida were also well distributed in these regions.

Further indication of the general occurrence of yeasts in the world's seas and oceans is the report by Kriss et al. (1955) of the discovery in the region of the North Pole of "pink" and "white" yeasts, budding forms of which were isolated from the silt of the bed of the Arctic Ocean at a depth of 3,450 metres. It thus appears that there is no northern limit to the occurrence of these organisms in the seas. Further recent investigations by Kriss (1962) in the Greenland Sea indicate that the yeasts there appear to be adapted mainly/

mainly to the deep layers of water, although none has been isolated from mud samples in the region. The full significance of this latter finding cannot be assessed until such time as data from microbiological and oceanographic studies can be compared.

Bhat and Kachwalla (1955) reported an investigation made of the yeast flora of water samples taken from the Indian Ocean, at an unspecified depth, two to six miles off the Indian coast, near Bombay. From seventeen samples, eighty yeast isolates were obtained, seventy-four of which were studied and assigned to seven genera as listed in Table 3.

TABLE 3

Species composition of yeasts collected two to six miles off the Indian coast, near Bombay - Bhat and Kachwalla (1955):-

Genus	Isolates	Species
Saccharomyces	10	S.Steineri, S.fructuum, S.rosei
Debaryomyces	8	D.hansenii, D.nicotiani, D.kloeckeri, D.subglobosus
Candida	30	C.tropicalis, C.guillermondii, C.melibiosi
Torulopsis	16	T.famata, T.glabrata, T.candida
Rhodotorula	6	Rh.pallida, Rh.mucilaginosa, Rh.minuta
Cryptococcus	2	Cr.laurentii
Trichosporon	2	Unidentified

The most common species found in the samples was Candida tropicalis, of which twenty-six isolates were obtained, i.e., approximately 36% of the total isolates. Also, as can be seen in Table 3, sporogenous yeasts have accounted for 24% of the total isolates.

In/

In 1958 an extensive investigation of the yeast flora of the estuarine waters of Biscayne Bay, Florida and adjacent benthic areas was started by a team of American workers - Fell et al. (1960). A selective enrichment culture technique was successfully employed and one hundred and seventy-nine yeast isolates (obtained from the waters of Biscayne Bay) were classified as in Table 4.

TABLE 4

Species composition of yeasts collected in Biscayne Bay, Florida - Fell et al. (1960):-

Species	Isolates	Species	Isolates
Debaryomyces kloeckeri	8	Cryptococcus albidus	1
Hansenula anomola	9	Rhodotorula mucilaginosa	24
Saccharomyces fructuum	2	Rhodotorula glutinis	13
Saccharomyces species	2	Rhodotorula texensis	5
Candida tropicalis	42	Rhodotorula minuta	4
Candida parapsilosis	18	Rhodotorula graminis	1
Candida guilliermondii	4	Rhodotorula species	1
Candida intermedia	4	Trichosporon cutaneum	10
Candida melinii	1	Torulopsis species	4
Candida boidinii	1	Pullularia pullulans	2
Cryptococcus laurentii	6	"black yeasts"	16

Candida tropicalis and Rhodotorula mucilaginosa were found to be the most abundant and widely distributed species in the Bay. The widespread occurrence of the former species agrees with the observa-:tions of Bhat and Kachwalla (1955) mentioned above.

Deep sea sediments from off the coast of the Bahamas were also examined by Fell et al. (1960), who found their yeast flora contained many of the same species isolated from Biscayne Bay, although the absence/

absence of Candida tropicalis in these samples is notable - see Table 5.

TABLE 5.

Species composition of yeasts collected from deep sea sediments in The Bahamas (Fell et al., 1960):-

Species	Isolates	Species	Isolates
Rhodotorula mucilaginosa	3	Candida tenuis	2
Rhodotorula glutinis	2	Candida curvata	5
Rhodotorula texensis	1	Candida guilliermondii	1
Rhodotorula marina	1	Cryptococcus diffluens	1
Debaryomyces kloeckeri	4	Cryptococcus albidus	3
Torulopsis famata	2	Cryptococcus neoformans	2
Candida parapsilosis	5		

Investigations of the yeast flora of sea-water, sediments and marine plants and animals were undertaken in the Californian coastal waters of the Pacific by Fell and van-Uden (1961) who found sporogenous and asporogenous yeast species to occur widely in these locations. It should be noted that asporogenous yeasts were generally predominant. Although no information was given concerning the numbers and types of yeasts isolated certain differences in species occurrence were noted by these workers between this collection and parallel collections made in Biscayne Bay, Florida and adjacent, deeper waters. For example, Candida tropicalis, C. parapsilosis and C.krusei, which are prevalent species in the latter areas are uncommon or absent in the Californian coastal waters. An interesting parallel to these observations is the failure of Fell (personal communication) to isolate from Florida coastal waters species of Metschnikowia which were isolated in/

in considerable numbers from the Pacific Ocean by van-Uden and Castelo-branco (1961) and which have also been detected in Clyde Estuary waters (see page 166). The above reports would appear to indicate variation in species occurrence in different geographical areas. Such variations may be attributed to minor differences in isolation media and techniques: however it seems more likely that the "microzonation" of yeasts described by Novozhilova (1955) may provide a more plausible explanation.

In a later study of the yeast flora of Florida coastal waters and deeper waters off The Bahamas, Roth et al. (1962) reported finding yeasts in 78% of the two hundred and twenty water and sediment samples examined by them. The most common yeasts encountered in these samples were species of Rhodotorula, Candida, Trichosporon, Debaryomyces and Cryptococcus. Candida tropicalis and Rhodotorula (mucilaginosa) rubra appeared to be the most common and widespread species in estuarine waters, whereas Candida parapsilosis and Debryomyces kloeckeri were most common in oceanic waters. Strains of black "yeast-like organisms" resembling Pullularia spp., were also isolated from all these areas.

Results of quantitative estimations of yeast populations in the above-mentioned locations indicate that in general yeasts occur in higher numbers in coastal than in pelagic waters. This observation is in general agreement with the findings of Novozhilova (1955). At a certain area, however, in the open waters of the Gulf Stream, samples taken from depths between 160 and 400 metres were found to contain yeasts/

yeasts in quantities (of 2,000 to 5,200 cells/litre sea-water) equalling or exceeding the average populations found in coastal waters. A similar species pattern, i.e., 60% Rhodotorula graminis, was demonstrated in all the samples taken between these depths and this differed entirely from that of the surface-water samples which contained almost exclusively Rhodotorula glutinis var. dairiensis (Hasegawa et al.1960) in numbers of only 200 cells/litre. This rich find of yeasts in the open ocean well below the surface can be compared to those already mentioned by Novozhilova (1955) and it seems in this case that the "microzoning" of the yeasts may well be due to the large phytoplankton and zooplankton content carried by the Gulf Stream. A similar phenomenon has been noted for heterotrophic bacteria in the oceans by Kriss et al. (1959) who found large populations of these organisms occurring at the interphases of large water masses.

The yeast flora of grasses and algae in the shallow regions of Florida coastal waters was also studied by Roth et al. (1962) and on the basis of frequency of yeast isolation, i.e., only 52% samples compared with 78% water samples demonstrated yeasts, and the fact that only a small number of species per sample were isolated, i.e., only one or two compared with six or more detected in the sea-water samples, these workers concluded that the yeast flora of these plants is only incidental to that of the surrounding sea-water. No cell counts in this instance were, however, undertaken to substantiate this conclusion.

The yeast flora of estuarine and brackish waters of the Miami River which flows into Biscayne Bay was investigated by Capriotti (1962)[B] /

(1962)[B] - see Table 6. He found that 60% of the species isolated were also found in Biscayne Bay by Fell et al. (1960) and Roth et al. (1962), e.g., Debaryomyces kloeckeri, Hansenula anomala, Candida tropicalis, C. melinii, Cryptococcus albidus, Rhodotorula glutinis and Aureobasidium pullulans.

TABLE 6

Order of frequency of species isolated in the Miami River area - Capriotti (1962)[B] :-

Species	Percentage Occurrence of Samples in which these species were found
Saccharomyces carlsbergensis	90.0
Candida tropicalis	31.0
Pichia fermentans	22.0
Torulopsis glabrata	18.0
Cryptococcus albidus	18.0
Hansenula anomala	9.0
Debaryomyces kloeckeri	9.0
Candida parapsilosis	9.0

The high incidence of S.carlsbergensis is notable here especially as this species was not detected in Biscayne Bay or in the off-shore waters. It is of interest also to note that of the species isolated by Capriotti (1962)[A] from soils in the region of the Miami River and Biscayne Bay, during another study, 80% were detected in the estuarine waters (Capriotti, 1962)[B] and 50% were also found in Biscayne Bay (Fell et al., 1960).

From the foregoing reports little information emerges which could elucidate the quantitative and qualitative inter-relationship between the/

the yeast flora of marine fish and other locations in the surrounding marine environment. However, one correlation would appear to be clear, namely, that each yeast species which has been reported isolated from fish in the few recent surveys cited has been found also in other marine sources in the same area and in some cases by other workers and in different geographical areas. Much more information is, however, required before the problem can be solved of whether yeasts found on fish form a commensal, parasitic or passively carried micro-flora. The solving of the latter problem may in turn help in the solution of other important problems, e.g., possible role of yeasts in fish spoilage, mechanism of yeast distribution in the seas, major sites of yeast reproduction in the seas, the role of yeasts in fish food chains, etc., thereby establishing the specific function of yeasts in the biological productivity of the seas.

This present investigation of the yeast flora of marine fish in Scottish coastal waters and oceanic waters arose initially from a study of a small number of yeast cultures isolated from fish and sent to this Department from the D.S.I.R. Torry Research Station, Aberdeen, and the Marine Station, Aberdeen. During this study, perusal of relevant literature then available (1959), although indicating the widespread occurrence of yeasts in the marine environment, also indicated how very meagre was the information, especially taxonomical, regarding yeast occurrence on fish. It was hence decided that a survey of marine yeasts, i.e., those isolated from a marine environment could/

could be undertaken profitably to provide more information with a view to forwarding the solutions of the problems cited above.

This thesis sets out the classical descriptions of isolates obtained during such a survey and also reports certain of their physiological and biochemical characteristics.

COLLECTION OF ISOLATES

Introduction

The literature survey indicates that when these studies were first contemplated little information was available regarding the requirements for isolating yeasts from marine materials. For example, little was known about suitable media, optimum pH, temperature, mineral salt concentrations, etc. In view of this lack of knowledge it was, therefore, considered expedient as an initial step to find a medium which would permit the isolation of a reasonable number of yeasts. At the same time it was realised that such a medium might not be suitable for the isolation of all the yeasts existing in the marine environment. However, at this stage of the investigation this latter point was not considered too great an obstacle.

The earlier publications also indicated that yeasts probably form a relatively small proportion of marine micro-florae. Hence direct plating techniques did not appear to offer a profitable line of approach. Accordingly it was decided to employ enrichment techniques although the limitations of such procedures were realised.

The first aim of this survey, therefore, was to obtain from marine material a reasonable number of yeasts from which information could be acquired regarding the types of yeasts, their distribution and physiological properties. Such knowledge, it was hoped, might then indicate better methods for isolation and further fields of study.

Methods

1) Choice of Isolation Medium:

The use of a suitably selective medium for the isolation of particular micro-organisms, or groups of such, from mixed populations, has proved an invaluable tool in studies of microbial ecology. Such media have been used successfully by many of the early workers before the last two decades for the purpose of isolating the bacterial flora from marine material. Unfortunately, these same workers, except for the few whose findings are mentioned in the introduction, disregarded or mentioned only briefly any occurrence of yeast colonies amongst those of the bacteria. Further, as few studies employing media specially selective for yeasts were undertaken during this period the erroneous idea that yeasts are not a specific part of the marine microflora was widely held until recently. It is interesting to note that Fischer (1894) used in his studies an isolation medium which somewhat favoured yeast growth in its nutrient composition and pH. Using these conditions he concluded that there are autochthonous yeast species in the seas, and in some of his samples yeasts were actually found to be the dominant type of flora.

The few workers who have made specific studies of marine yeasts employed a variety of selective media. Nadson and Burgwitz (1931) used malt wort supplemented with 3% NaCl, while McCormack (1956) used Sabouraud's agar acidified with lactic acid. Meat-peptone agar, fish-peptone agar and plain wort agar made with tap- or sea-water, were/

were all used by Kriss (1959). Phaff et al. (1952) isolated most of their strains on potato-dextrose agar at pH 3.5. A basal medium of inorganic salts, including 2% NaCl, in distilled water and supplemented with glucose and sodium nitrate, or sucrose and ammonium sulphate, was employed by Bhat and Kachwalla (1955). It thus appeared that many media capable of supporting growth of terrestrial yeasts could with slight modifications be used to promote the growth of those from marine habitats.

The medium "MYGP" devised by Wickerham (1951) has proved an excellent medium for the cultivation of yeasts by providing adequate sources of carbon, nitrogen, sulphur, minerals and certain nutrilites (Morris, 1958) and has the following composition:-

"MYGP" Medium

Yeast extract	0.3% w/v
Malt extract	0.3% w/v
Peptone	0.5% w/v
Glucose	1.0% w/v
Distilled water	to 100.0%

(pH of approx. 5.5)

It was decided to use this medium as the basis of a selective medium because the fish yeasts held in this department prior to this investigation were found to thrive on it.

It was noted that these fish yeasts grew well in "MYGP" to which no salts or sea-water were added. Such supplements were made, however, by Kriss (1959), Bhat et al. (1955), Nadson and Burgwitz (1931) in their/

their yeast isolation media. No distinct requirement or preference for sea-water by marine yeasts was demonstrated by these workers but as such a requirement has been found in freshly isolated marine bacteria by Zobell and Upham (1944), Zobell (1946), Wood (1953), Liston (1956) and MacLeod and Onofrey (1956) it was decided to include sea-water in the yeast isolation medium. Sea-water, free from terreginous contamination, was therefore obtained from the North Sea and in accordance with the recommendations of Zobell (1946) it was allowed to "age" by storage in glass bottles in the dark for some weeks prior to usage. This "ageing" process was said by Zobell to stabilize the organic content of the water thereby giving more reproducible results and also to decrease the slightly bacteriostatic principle present in raw sea-water. Before use the "aged" sea-water was diluted, three parts with one part distilled water, a practice found to give improved results for bacterial isolations (Hobbs, personal communication).

In choosing a selective medium for this investigation of fish yeasts it was desirable to have one which would allow the cultivation of the yeast flora and at the same time, it was hoped, one which would not be too selective within this flora, that is, favour the growth of a particular species of yeast. The use of antibiotics as an aid in isolations was considered initially but in the light of evidence of some antagonism to yeast reproduction by certain antibiotics - Beech and Carr (1955) and Morris (personal communication) - it was decided not to employ these agents until further investigations could be carried out/

out. The possible danger of using antibiotics is further supported by the recent findings of di Menna (1962) and Krasil'nikova (1962) who found that yeast growth could be inhibited by antibiotics produced by cultures of bacteria, Streptomyces, Actinomyces and moulds.

The ability of yeasts to reproduce best in media of pH range within 3.0 to 6.0 is a well established fact and has often been exploited in isolating yeasts from a mixed population containing bacteria since many of the latter are unable to develop satisfactorily below approximately pH 5.5. Phaff et al.(1952) used a pH value of 3.5 for most of their isolations and McCormack (1956) successfully separated yeasts from bacteria on a medium acidified with lactic acid. The value of 5.5 was decided upon for the medium to be used in the preliminary studies of the present survey because it is about the average optimum pH value for most yeasts and is the value at which growth of many bacteria is suppressed.

Accordingly, in the first collection of samples a selective medium of the following composition was employed:-

"MYGP5" Medium

Yeast extract		0.3% w/v
Malt extract		0.3% w/v
Peptone		0.5% w/v
Glucose		1.0% w/v
+ Aged sea-water, 3 parts) Distilled water, 1 part)	to	100.0%

pH 5.5

The/

The medium which will be referred to as "MYGPS", distributed into half-ounce bottles (screw-cap), was autoclaved at 10 lb/square inch pressure for twenty minutes.

2) Isolation Temperature:

In northern waters, where the fish sampled in this investigation were caught, the sea temperatures vary from -2°C to 12°C. Bacterio-:logical studies of the seas, reported by Zobell (1959), have, however, indicated that the optimum temperature for the multiplication of most marine bacteria, including those from the cold bottom deeps, is 18°C to 22°C. Liston (1956) and Georgala (1958) in their studies of the bacterial flora of flatfish and North Sea cod, respectively, found higher counts at 22°C than at 0°C, although occasionally higher counts were obtained at the lower temperature.

In their isolations of marine yeasts Phaff et al. (1952) used a temperature of 30°C, Kriss and Novozhilova (1954) 18°C to 26°C and Kriss et al. (1955) 25°C to 30°C. Although yeasts have been found to reproduce over a wide range of temperatures, as indicated by the findings of Phaff et al.(1952) and Lund (1954), who found yeasts able to reproduce within the range 0°C to 38°C with optimal reproduction (rate and amount) occurring in the range 20°C to 30°C, there is evidence of the existence of psychrophilic yeast strains. Several reports of the occurrence of yeast strains in non-marine habitats have/

have been published, e.g., Scott (1937) isolated strains resembling Trichosporon scottii from chilled beef which were unable to reproduce above 25°C and exhibited optimal growth below 20°C; Lawrence et al. (1959) found Candida strains in chilled grape juice exhibiting optimal reproduction at approximately 11°C and which were unable to reproduce above 21°C; Straka and Stokes (1960) isolated a yeast from glacier ice in Antarctica which they stated resembled Candida strains described by Lawrence et al., these reproduced between 7°C and 20°C having an optimum in the range 5°C to 10°C; diMenna (1960) isolated strains of Candida scottii unable to grow above 15°C from soil samples also taken in Antarctica, in McMurdo Sound. In view of this evidence, the possibility of the general occurrence of psychrophilic yeasts in these northern waters is almost certain. It is of interest to note here that during these present studies the only sea-water sample incubated at 4°C yielded a yeast strain with an optimum growth temperature in the range 14°C to 18°C and which grew only slightly at 25°C.

In view of the findings of these workers, reported above, it was decided to carry out isolations in the present study at 25°C, thus facilitating a comparison of results obtained in this survey with those of other reports on this topic. However, it is borne in mind that psychrophils do exist and that the study of these could provide a topic for future investigation.

3) Sampling of the Fish:

Bacteriological investigations by Stewart (1932/33), Dyer (1947), Shewan/

Shewan (1949) (1961), Tarr (1954), Liston (1957, 1956), Georgala (1957), (1958) and many other workers, of the micro-flora of live or freshly caught fish have indicated that large microbial populations exist in the slime layer covering the skin, in the gills and also in the intestine of "non-fasting" fish. Fasting fish have been found by Margolis (1953) to have sterile intestinal tracts - an indication that there may be no true intestinal flora in these fish and the organisms detected there have been introduced with ingested food. It is also generally accepted that the internal organs, body fluids and muscle tissue of these fish are sterile - Liston (1956), Shewan (1961), Dyer (1947), Georgala (1957), Tarr (1954) - although some workers have disagreed with this, e.g., Bissett (1948) who found bacteria in the muscle of fresh-water fish. It is very possible, however, that the latter worker had detected pathogenic organisms.

In view of these findings it was decided that, in general, the samples should be taken from the following areas on the fish - slime layer, gills and intestinal contents.

Collections

1) First Collection - Clyde Estuary, 4th May, 1960:

Initially it was intended to catch the fish by lines with baited hooks, a method employed commercially to catch large cod (Gadus callarias) and/

and halibut (Hippoglossus hippoglossus)in certain fishing grounds off the west coast of Scotland (Hardy, 1959). In an investigation of fish micro-flora, such as this one, line-catching is certainly the method of choice as the fish are caught individually and would be subjected to little or no handling or rough usage prior to being sampled. The necessary gear for this purpose was not,however, available and further, in the limited time available for sampling, line-catching might have proved too slow and tedious to allow examination of a range of fish species. It was decided, therefore, to catch fish by trawl-net. In trawling, excellent descriptions of which have been written by Cutting (1955) and Hardy (1959), the fish in the net are swept along the sea-:bed and bumped against each other, the net, ropes, etc., before being hauled to the surface and allowed to spill onto the deck of the vessel from the "cod-end", i.e., the bottom end of the bag. It is obvious that such treatment could appreciably alter the skin micro-flora from that present in the normal, free-swimming fish, as organisms will be picked up from the sea-bed as well as from the skins and expressed gut-:contents of other fish in the trawl-net. Shewan(1949) found bacterial loads to be ten to a hundred times heavier from the skins of trawled than line-caught fish.

Two "hauls", i.e., catches by trawl-net were made on 4th May 1960, off the Cumbraes, in the Clyde Estuary, at a depth of 40/60 metres. Twenty-seven fish from the catches which had received the minimum of handling were sampled as soon as possible after landing on the ship. Sterile throat swabs were rubbed over the skin, pushed into/

into the gills through the gill-openings or had the gut contents expressed onto them through the anus. Immediately after taking the sample the swabs were dipped into the bottles containing the isolation medium, shaken slightly and quickly withdrawn.

Altogether, sixty-six samples were taken from twenty-seven fish of seven different species - see Table 7. The samples were then brought to the laboratory as quickly as possible and incubated at 25°C. The time interval between being taken and reaching the laboratory was six hours for samples Nos. 1 to 30, taken from the first "haul" and three hours for Nos. 31 to 66, from the second "haul". No special precautions regarding temperature control of the samples during transportation were taken but at no time did the atmospheric temperature rise above 25°C.

After forty-eight hours at 25°C three sub-cultures were made from each sample into fresh "MYGPS" at pH 4.0, 3.5 and 3.0, respectively. The cultures were observed daily for visible growth and after a week microscopical examination revealed yeast cells mainly in the original cultures of the samples and the secondary cultures at pH 4.0. From the four cultures of each sample, that at the lowest pH exhibiting yeast growth was chosen for further study since this was the least likely to have excessive bacterial contamination. Sub-cultures were made onto petri dishes containing "MYGPS" in 2% agar-agar at pH 5.0.

The plates were incubated at 25°C for two to three days when single/

TABLE 7

First collection of samples from fish caught at a depth of approx. 40-60 metres by trawl-net off the Cumbraes in the Clyde Estuary, 4/5/60.

Sample No.	Fish	Area of Sampling	Sample No.	Fish	Area of Sampling
1	Haddock A	Gills	36	Skate B	Faeces
2 ×	"	Faeces	37	Skate C	Skin
3	Haddock B	Gills	38	"	Gills
4	"	Faeces	39 ×	"	Faeces
5	Haddock C	Gills	40	Skate D	Skin
6	"	Faeces	41 ×	"	Gills
7 ×	Haddock D	Gills	42 ×	"	Faeces
8 ×	"	Faeces	43 ×	Haddock E	Skin
9	Cod A	Gills	44	"	Gills
10	"	Faeces	45	"	Faeces
11 ×	Cod B	Gills	46 ×	Haddock F	Skin
12	"	Faeces	47	"	Gills
13 ×	Cod C	Gills	48	"	Faeces
14	"	Faeces	49 ×	Haddock G	Skin
15 ×	Cod D	Gills	50 ×	"	Gills
16	"	Faeces	51	"	Faeces
17	Saithe A	Gills	52 ×	Haddock H	Skin
18	"	Faeces	53 ×	"	Gills
19	Hake A	Gills	54	"	Faeces
20	"	Faeces	55	Cod E	Skin
21 ×	Plaice A	Gills	56	"	Gills
22	"	Faeces	57	"	Faeces
23	Plaice B	Gills	58	Cod F	Skin
24 ×	"	Faeces	59 ×	"	Gills
25	Plaice C	Gills	60	"	Faeces
26	"	Faeces	61	Lemon sole B	Gills
27	Plaice D	Gills	62 ×	"	Faeces
28	"	Faeces	63 ×	"	Skin
29	Lemon sole A	Gills	64	Lemon sole C	Skin
30	"	Faeces	65 ×	"	Gills
31 ×	Skate A	Gills	66 ×	"	Faeces
32	"	Skin	67 ×	------	Deck
33	"	Faeces	68 ×	------	Trawl-net
34 ×	Skate B	Skin			
35	"	Gills	69	------	Deck

× Samples from which a yeast culture was obtained

See Table 15 for the Latin designations of the fish listed above

single colonies were removed. Only one representative colony of similar colonial types occurring on a plate was selected and this was replated through Ringer's solution. On obtaining pure cultures, i.e., identical colonies, a single colony was removed and streaked on slopes of the same medium held in one-ounce, screw-cap bottles. After incubation for two to three days at 25°C these pure yeast cultures were stored at 4°C awaiting examination.

Yeast strains were detected on eighteen of the twenty-seven fish sampled, i.e., 66%. Altogether twenty-four cultures were obtained from the sixty-six fish samples from the various areas on the fish, i.e., 41%. As only one representative colony of identical morpho-:logical types occurring on the plates was removed - a measure considered expedient since it is not practicable to handle vast numbers of cultures which would otherwise be obtained - a qualitative rather than a quantitative estimation of the yeasts present in the fish samples has been obtained. These figures represent the percentage of strain occurrence in the samples but cannot, of course, be regarded as an absolute estimation because yeast strains having the same colonial and morphological appearance but differing in other characteris-:tics may well have been overlooked.

2) Second Collection - Clyde Estuary, 27th June, 1960:

A second collection of samples was made on 27th June, 1960 from two "hauls" taken in the Clyde Estuary and on this occasion ninety-eight samples were taken from thirty-eight fish - see Table 8.

TABLE 8

Second collection of samples from fish caught at a depth of approx. 40-60 metres, Fintry Bay, Clyde Estuary (nos. 101-150) and at 60-80 metres, off Mt. Stewart, Clyde Estuary, (nos. 151-198), 27/6/60:

Sample No.	Fish	Area of Sampling	Sample No.	Fish	Area of Sampling
101	Haddock A	Gills	150 x	Plaice F	Skin
102 x	"	Faeces	151	Skate A	Skin
103 x	Haddock B	Gills	152	"	Gills
104 x	"	Faeces	153	"	Faeces
105	Haddock C	Gills	154	Whiting A	Gills
106	"	Faeces	155	"	Skin
107 x	Cod A	Gills	156	"	Faeces
108 x	"	Faeces	157 x	Whiting B	Gills
109	Cod B	Gills	158 x	"	Skin
110	"	Faeces	159 x	"	Faeces
111	Cod C	Gills	160 x	Whiting C	Gills
112 x	"	Faeces	161 x	"	Skin
113	Plaice A	Gills	162	"	Faeces
114	"	Faeces	163	Witch A	Gills
115 x	Plaice B	Gills	164 x	"	Skin
116	"	Faeces	165 x	"	Faeces
117 x	Plaice C	Gills	166 x	Witch B	Gills
118	"	Faeces	167 x	"	Skin
119	Plaice A	Skin	168	"	Faeces
120 x	Plaice B	Skin	169 x	Witch C	Gills
121	Plaice C	Skin	170 x	"	Skin
122	Lemon sole A	Gills	171	"	Faeces
123	"	Faeces	172	Cod D	Gills
124	"	Skin	173	"	Skin
125	Lemon sole B	Gills	174	"	Faeces
126	"	Faeces	175 x	Cod E	Gills
127	"	Skin	176 x	"	Skin
128	Dogfish A	Gills	177 x	"	Faeces
129	"	Faeces	178	Cod F	Gills
130 x	Dogfish B	Gills	179 x	"	Skin
131 x	"	Faeces	180	"	Faeces
132 x	Dogfish C	Gills	181 x	Saithe A	Gills
133	"	Faeces	182 x	"	Skin
134 x	Gurnard A	Gills	183 x	"	Faeces
135	"	Faeces	184	Hake A	Gills
136 x	Haddock D	Gills	185 x	"	Skin
137	"	Faeces	186	"	Faeces
138	Haddock E	Gills	187 x	Whiting D	Gills
139	"	Faeces	188 x	"	Skin
140	Haddock F	Gills	189 x	"	Faeces
141	"	Faeces	190 x	Whiting E	Gills
142	Haddock G	Gills	191 x	"	Skin
143	"	Faeces	192 x	"	Faeces
144	Plaice D	Gills	193 x	Whiting F	Gills
145	"	Faeces	194 x	"	Skin
146 x	Plaice E	Gills	195 x	"	Faeces
147 x	"	Faeces	196 x	Whiting G	Gills
148 x	Plaice F	Gills	197 x	"	Skin
149	"	Faeces	198	"	Faeces

x Samples from which a yeast culture was obtained.

See Table 15 for the Latin designations of the fish listed above.

Samples Nos. 101 to 150 were taken from the first "haul" made at 40/60 metres in Fintry Bay, Great Cumbrae and Nos. 151 to 198 were from the second "haul" made at 60/80 metres off Mount Stuart, Bute. The same sampling methods were employed as in the first collection except that the pH of the isolation medium "MYGPS" was lowered to 5.0 and wooden swabs, which were broken from their holders and left in the medium, were used to take thirty-two of the samples (Nos. 166/198) in place of the wire-dip ones. These wooden swabs had to be removed after three to four days as moulds were found to be growing on the broken stalks in many of the cultures.

The inclusion of mould-inhibiting agents in the isolation medium was considered in view of this mould growth. Hertz and Levine (1942) found 100 p.p.m., diphenyl in malt extract agar inhibited a large percentage of pure mould cultures and only a few yeast species, while Mrak and Phaff (1948) found that a 5% wort agar with 2,500 p.p.m., sodium propionate was quite effective in separating yeasts and moulds. Etchalls et al. (1954) found, however, that both diphenyl and sodium propionate could be inhibiting to certain yeast species and strains and did not recommend their use for total population yeast isolations. In view of these findings and the slow rate at which moulds developed in the isolation medium it was decided not to add any mould-inhibiting agents.

The samples from the first and second "hauls" reached the laboratory six and three hours, respectively, after being taken and, as/

as before, the atmospheric temperature during transportation did not rise above 25°C.

Secondary cultures were prepared in "MYGPS" at pH 4.0, 3.5 and 3.0 from the original cultures which had been incubated at 25°C for sixty-two hours and pure cultures were obtained by plating procedures as described for the first collection. Altogether forty-eight isolates were obtained from the ninety-eight samples, i.e., 49% occurrence and yeasts were found on twenty-eight of the thirty-eight fish sampled, i.e. 73%.

Better results were obtained with wooden swabs. As can be seen in Table 8, twenty-two representative cultures were isolated from thirty-two samples taken with wooden swabs, i.e., 68% occurrence, whereas only twenty-six representative cultures were isolated from the sixty-five wire-dip samples, i.e., 40% occurrence. The increase in the number of yeast strains isolated using the wooden swabs may arise from the longer contact of the swab in the medium when any yeast cells present in the sample have time to establish growth before being removed, as may well happen with the wire-dip swabs. Also, the presence of organic materials from the sampled area, i.e. fish slime, faeces, etc., absorbed onto the swab may help to promote the growth of the yeast cells.

3) Third Collection - North Sea, off Aberdeen, 19th July, 1960:

A trawl-net was used to catch the fish as special equipment for line-catching/

line-catching was not available. Only one "haul" was made on this occasion at 64 metres, three to four miles off the Aberdeenshire coast, some miles south of Aberdeen, from which twenty-eight fish were sampled. Samples were also taken in this instance from the mouths of the fish, through which large amounts of sea-water pass to the gills in the free-swimming fish.

In view of the increase in the numbers of yeast types isolated in the last collection using wooden swabs these were employed for all sampling here. On reaching Aberdeen two hours after taking the samples, the cultures were placed at 25°C in the Torry Research tation. These were removed from the incubator about sixteen hours later for transportation to Glasgow, where, twenty-four hours later, they were placed at 25°C. Once again, during transportation, the atmospheric temperature did not rise above 25°C.

Altogether one hundred and four samples were taken from twenty-eight fish (see Table 9). Samples Nos. 200 to 249 were placed in isolation medium, i.e., "MYGP" at pH 5.0, while Nos. 301 to 371 were placed in that of pH 4.0. As can be seen in Table 9, the number of yeast types isolated in the medium at pH 4.0, i.e., fifty from fifty-four samples - 92% - is much higher than that at pH 5.0, i.e., thirty from fifty samples - 60%. Less bacterial contamination was observed also in the samples placed in the medium at pH 4.0. Occasionally the long rod-shaped and large round bacteria which were common forms in the medium/

TABLE 9

Third collection of samples from fish caught at a depth of 64 metres, 3-4 miles off the Aberdeenshire coast, 19/7/60:-

Sample No.	Fish	Area of Sampling	No. of Strains
200	Dab A	Gills	—
201	"	Faeces	—
202X	Haddock A	Skin	1
203	"	Gills	—
204X	"	Mouth	1
205	"	Faeces	—
206	Haddock B	Skin	—
207X	"	Gills	1
208X	"	Mouth	1
209X	"	Faeces	1
210	Haddock C	Skin	—
211	"	Gills	—
212X	"	Mouth	1
213X	"	Faeces	1
214X	Whiting A	Skin	1
215X	"	Gills	1
216	"	Mouth	—
217	"	Faeces	—
218	Whiting B	Skin	—
219X	"	Gills	1
220	"	Mouth	—
221	"	Faeces	—
222X	Whiting C	Skin	1
223	"	Gills	—
224X	"	Mouth	1
225	"	Faeces	—
226X	Haddock D	Skin	1
227X	"	Gills	1
228X	"	Mouth	1
229	"	Faeces	—
230X	Cod A	Skin	2
231X	"	Gills	1
232	"	Mouth	—
233X	"	Faeces	1
234	Gurnard A	Skin	—
235X	"	Gills	1
236X	"	Mouth	1
237	"	Faeces	—
238X	Gurnard B	Skin	1
239	"	Gills	—
240X	"	Mouth	1
241	"	Faeces	—
242X	Mackerel A	Skin	1
243	"	Gills	—
244	"	Mouth	—
245X	"	Faeces	1
246X	Hake A	Skin	2
247	"	Gills	—
248X	"	Mouth	2
249X	"	Faeces	1
301X	Lemon Sole A	Gills	1
302X	"	Skin	2
303X	Lemon Sole A	Faeces	1
304X	"	Mouth	1
305X	Lemon Sole B	Skin	1
306X	"	Gills	1
307X	"	Faeces	1
308X	"	Mouth	2
309X	Lemon Sole C	Skin	2
311X	"	Gills	1
312	"	Faeces	—
313	"	Mouth	—
314X	Lemon Sole D	Skin	1
315	"	Faeces	—
316X	"	Mouth	1
317X	Plaice A	Skin	2
318X	"	Gills	1
319X	"	Mouth	1
320X	"	Faeces	1
321X	Plaice B	Skin	2
322X	"	Gills	1
323	"	Mouth	—
324	"	Faeces	—
325X	Cod B	Mouth	1
326	"	Gills	—
327X	"	Skin	1
328	"	Faeces	—
329X	Mackerel B	Skin	2
330X	"	Gills	2
331X	"	Mouth	1
332X	"	Faeces	1
333X	Mackerel C	Skin	1
334	"	Gills	—
335X	"	Mouth	1
336X	"	Faeces	1
337X	Mackerel D	Skin	1
338X	"	Gills	1
339	"	Mouth	—
340X	"	Faeces	1
341X	Dab B	Skin	2
342X	"	Gills	1
343X	"	Faeces	1
344X	Dab C	Skin	1
345X	"	Gills	1
346	"	Faeces	—
347X	Dab D	Skin	1
348X	"	Gills	1
349	"	Faeces	—
350	Dab B	Skin	—
354X	Plaice C	Gills	2
360X	"	Skin	1
361	"	Faeces	—
370X	Plaice D	Gills	1
371X	"	Skin	1

See Table 15 for the Latin designations of the fish listed above.

X Samples from which a yeast culture was obtained

medium of higher pH were found to develop, even in the media of lower pH, e.g., 4.0, 3.5 and 3.0. In view of the increase in the numbers of yeasts isolated at pH 4.0 this value was chosen for future isolations.

As on the previous occasion secondary cultures were prepared at pH 4.0 (Nos. 200 to 249 only), 3.5 and 3.0, and pure cultures eventually obtained. Again, only one representative colony was taken from a plate and altogether eighty yeast isolates were obtained from the one hundred and four samples taken, i.e. 77%. This is much higher than the corresponding figures in the first two collections, i.e., 41% and 49%, respectively. It is, however, difficult to account for the increase in the number of types obtained here. There may be several contributory factors in this instance, e.g., location of catching the fish, seasonal variation in the fish micro-flora and improvements in sampling and isolation techniques, e.g., lower pH of medium and the use of wooden swabs.

It is also of interest to note here that yeasts were isolated from all the twenty-eight fish sampled.

4) Fourth Collection - Gletty Bank, off the East Coast of Iceland, 11th and 14th September, 1961:

Samples were taken from fish caught by trawl-net at the south edge of the Gletty Bank, off the East Coast of Iceland, at 210/220 metres. Isolation medium "MYGPS" at pH 4.0 and wooden swabs were employed, as previously. Fifteen samples were taken altogether from the gills, skin and intestinal contents of fourteen fish (see Table 10).

TABLE 10

Fourth collection of samples from fish caught at a depth of 210/220 metres, south edge of the Gletty Bank, East Coast of Iceland, on 11th and 14th September, 1961:-

Sample No.	Date of Sampling	Fish Sampled	Area of Sampling
417 x	11/9/61	Witch A	Skin
418 x	"	Saithe A	"
421 x	"	"	Gills
420 x	"	Cod A	Skin
423 x	"	Cod B	Gills
422 x	"	Haddock A	Skin
429 x	"	Haddock B	Gills
424 x	14/9/61	Soïe A	Skin
425 x	"	Bream A	"
419 x	"	Bream B	"
427 x	"	Bream C	Gills
428 x	"	Lemon sole B	Faeces
450 x	"	Cod C	"
443 x	"	Haddock C	"
444	"	Bream B	"

(See Table 15 for the Latin designations of the fish listed above.)

x Samples from which a yeast culture was obtained

The swabs were removed from the medium after three to four days to prevent possible mould growth on the stalks, as found before. The cultures were held between 17°C and 20°C during the remainder of the voyage and on arrival at Aberdeen were dispatched on 21st September, 1961 to Glasgow where they arrived on 26th September, 1961. The temperature at which the cultures were held while in the post is not known, but an immediate microscopical examination revealed the presence of yeast cells in nearly all of them with little or no bacterial growth apparent. The cultures were, therefore, immediately seeded onto plates of "MYGPS" containing 2% agar-agar at pH 5.0 and secondary/

secondary cultures at pH 3.5 and 3.0 were also inoculated.

After two to three days at 25°C good growth of yeast colonies - in some cases two or three distinct colonial types (see Figure 1) were apparent on one plate - was found on thirteen of the fifteen seeded plates, only three of which were contaminated with bacteria. Pure cultures from single representative colonies from each plate were obtained as before.

FIGURE 1 Yeast colonies growing on "MYGPS" agar - sample 423.

Simultaneously with the seeding of the original cultures onto the solid medium at pH 5.0 broths of isolation medium "MYGPS" at pH 4.0, containing a mixture of antibiotics were also inoculated and placed at 25°C for two to three days. The latter medium was prepared by making sterilised "MYGPS" at pH 4.0 up to volume with a filter-sterilised/

sterilised solution (aqueous) of a mixture of antibiotics in sufficient quantity to give final concentrations in the medium of 10mg% aureomycin, 2mg% chloramphenicol and 2mg% streptomycin sulphate. Fell et al. (1960) isolated a broad range of yeast species by the inclusion of such an antibiotic mixture in their isolation media. Although as stated earlier, antibiotics have in some instances been found to suppress yeast growth it was hoped to ascertain the effects of these agents on the type of flora being isolated in these studies by comparing the growth obtained using plain isolation media and that supplemented with the antibiotics.

Plates of plain isolation medium at pH 5.0 were inoculated from broths containing the antibiotics and placed at 25°C for two to three days when comparison with the first set of plates inoculated from the original cultures was possible. A comparison was possible only by assuming that identical colonial types on the two plates from each sample represent the same yeast strain - see Table 11. These results indicate that the antibiotic medium was probably superior to the plain one inasmuch as seventeen other distinct colonial types were found as well as strains (as represented by identical colonies) which were common to both media. Also on the former medium the bacteria present in samples 425, 428 and 429 failed to develop.

It is difficult to explain why more yeasts developed in the antibiotic medium. The antibiotics themselves may have exerted an effect either in stimulation of these yeasts or in causing slight suppression/

TABLE 11

Yeast cultures from the fourth collection

Culture No.	Growth on plain "MYGPS" plates	Growth on "MYGPS" plates plus antibiotics
417m	+	+
417s	-	+
417p	+	+
418	+	+
419m	+	+
419s	-	+
419H	-	+
420m	+	+
420s	+	+
420H	-	+
421s	+	+
421m	-	+
421H	-	+
422m	+	+
422s	+	+
422p	-	+
423m	+	+
423s	-	+
423p	+	+
424s	+	+
424H	-	+
424p	+	+
425	+	+
427m	+	+
427s	-	+
427r	+	+
427p	+	+
428w	+	+
428p	+	+
429	+	+
443w	-	+
443p	-	+
450w	+	+
450p	+	+

suppression of the types dominating the flora, to the advantage of the others. Another explanation could be that the antibiotics, by suppressing the growth of bacteria, prevented the accumulation of yeast inhibitors which may be produced by the bacteria in the control cultures. In view of this increase in the numbers of representative yeast strains obtained by the inclusion of antibiotics in the isolation medium it was decided to test the whole yeast collection on the above antibiotic medium for any possible inhibitory effects. Although these agents have been used by some workers for marine yeast isolations - Fell et al. (1960) and Capriotti (1962)[A] - no information exists regarding their effect on the growth of yeasts isolated without antibiotics. No inhibition of growth was, however, noted for any strain in the collection but it must be remembered that most of these strains have been isolated in the one particular medium, "MYGPS", which may select strains which are not sensitive to antibiotics and, until broader tests of isolation media have been made, the author would recommend the use of both anti-biotic-containing and plain media for the isolation of marine yeasts.

The extra colonies, i.e., strains, were plated out for pure cultures as before. Altogether from the fifteen samples taken thirty-four yeast strains were obtained and of the fifteen fish sampled yeasts were detected on fourteen - see Table 11.

5) Line-Caught Fish - East of Aberdeen, 19th and 20th April, 1961:

On the 19th and 20th of April, 1961 attempts were made from the DSIR trawler "Sir William Hardy" to catch fish by lines. This proved to/

to be a difficult task as the trawler was not equipped for this type of fishing but after "shooting" the lines three times for approximately two hours on each occasion four fish were caught. These were lifted out of the water without contact with the vessel and samples taken from their gills, skins, mouths and intestinal tracts.

TABLE 12

Samples from fish caught by lines at 74 metres on 19th and 20th April, 1961:-

Sample No.	Date of Sampling	Fish Sampled	Position of catching fish	Area of fish Sampled
401	19/4/61	Turbot A	North Sea, 51 miles, East of Aberdeen	Skin
402 X	"	"	"	Mouth
403	"	"	"	Gills
404	"	"	"	Faeces
405 X	"	Ling A	"	Skin
406	"	"	"	Mouth
407 X	"	"	"	Gills
408	"	"	"	Faeces
409	20/4/61	Skate A	North Sea, 31 miles east of Aberdeen	Skin
410	"	"	"	Mouth
411	"	"	"	Gills
412	"	"	"	Faeces
413	"	Skate B	"	Skin
414	"	"	"	Mouth
415	"	"	"	Gills
416	"	"	"	Faeces

Samples from which cultures were obtained:- X

See Table 15 for the Latin designations of the fish listed above.

Isolation medium, "MYGFS", at pH 4.0 and wooden swabs were used in/

in the sampling. As can be seen in Table 12 a turbot - Scopthalmus maximus - and a ling - Molva molva - were caught on 19th April, 1961 at 74 metres, 51 miles east of Aberdeen and on 20th April, 1961 two skate - Raja species - were caught 44 miles east of Aberdeen at 74 metres. Altogether sixteen samples were taken from these fish and the cultures dispatched to Glasgow where an immediate microscopical examination revealed yeast cells present in at least seven of them. All were seeded onto plates of "MYGPS" at pH 5.0 and broths of "MYGPS" at pH 4.0,3.5 and 3.0 were inoculated for secondary cultures. Unfortunately, heavy mould growth was found to be present in most of the cultures when they arrived in Glasgow and this contamination greatly hindered isolation. Pure yeast cultures were obtained from only three of the original cultures of the samples, Nos. 402, 405 and 407 (see Table 12).

6) Cultures of Miscellaneous Origin held in the Collection:

Besides the one hundred and eighty-nine yeast strains isolated in these five collections thirty-three strains from various marine sources, including fish, have been sent to this laboratory by different workers and held with the former strains for classification and other studies.

The largest single group of such strains was sent by Dr. N.Smith of the Scottish Home Office Marine Laboratory, Aberdeen, who isolated them in March,1958 from salmon - Salmo salar - returning from the sea to spawn and caught at Park, 14 miles up the River Dee, Aberdeenshire.

In/

In sampling the fish the latter worker placed swabs from the gills and slime layer in sterile test-tubes and later streaked plates of Czapek-Dox medium made with either fresh- or sea-water(see Table 13).

TABLE 13

Yeasts from salmon, River Dee, Park, Aberdeenshire, March 1958:-

Culture No.	Swab No.	Source	Solvent used for Isolation Medium	Isolation Temperature
Y3	I	Gills	FW - fresh-water	22°C
YII	I	"	FW	"
Y54	V	"	FW	"
254	I	"	SW - sea-water	"
279	V	"	SW	"
281	V	"	SW	"
431	V	Slime	SW	"
1108	XIII	"	FW	"
1109	"	"	FW	"
1110	"	"	FW	"
1111	"	"	FW	"
1112	"	"	FW	"
1113	"	"	FW	"
1114	"	"	FW	"
1131	XIV	"	FW	"
1301	XIII	"	SW	"
BY1	I	"	SW	37°C
BY2	I	"	SW	"

See Table 15 for the Latin designation of the fish listed above.

Altogether, as indicated in Table 13, eighteen yeast strains were isolated sixteen of which were found in cultures incubated at 22°C and the other two, both black yeasts, which were later identified as Pullularia pullulans, were found after incubation at 37°C.

Nine of these isolates - 281, 431, BY1, BY2, Y3, Y11, Y54, 254, and 279 - were sent to this laboratory prior to this investigation for classification and study and they were maintained during this time on plain "MYGP" agar slopes (Morris, personal communication). The other nine strains were held at Torry Research Station, Aberdeen, on Czapek-Dox medium until they were dispatched to this laboratory in February 1960.

Strains T2, T4, T6, T7, 846 and 845 which were isolated from marine fish during bacteriological studies at Torry Research Station were also held on plain "MYGP" medium in this laboratory prior to the present investigation. Strains 846 and 845 were studied and classified (Morris personal communication). The sources of these strains are indicated in Table 14.

Strains 40red, T40, 12pink, Light I, and Light II - the sources of which are also indicated in Table 14 - were sent to this laboratory by Dr. A.D.Floodgate, Torry Research Station, in February 1960, on Czapek-Dox medium. Light I and Light II were isolated from a Winogradsky column(Larson,1954) using seaweed instead of straw.

Also indicated in Table 14 are strains 43/6 and 1174 which were isolated by Dr.I.Anderson, Anderson College, Department of Bacteriology, Glasgow University, during his studies of the bacterial flora of seawater in the North Sea off the Aberdeenshire coast.

TABLE 14

Source of miscellaneous cultures of marine origin:-

Culture No.	Source	Place or Person from which/whom received
T2	Cod - faeces	Torry Research Station, Aberdeen.
T4	"	"
T6	"	"
T7	"	"
846	Skate- gills	"
845	"	"
40red	Winkle in rock pool	"
T40	"	"
12pink	Decaying Laminaria	"
Light I	Seaweed in Winogradsky column	"
Light II	"	"
43/6	Seawater, North Sea, 10 miles off Aberdeen, at 12 metres.	Dr. I. Anderson, Glasgow University.
117A	"	"
HER3	Decomposing smoked herring	Torry Research Station, Aberdeen.
HER7	"	"

See Table 15 for the Latin designations of the fish listed above.

All the above mentioned strains after a purity check were trans-:ferred to slopes of "MYGP5" at pH 5.0 and maintained at 4°C with two to three monthly transfers to fresh media.

7) Sea-water Isolates - Clyde Estuary, 28th August, 1961:

As can be seen from literature reviewed in the Introduction the occurrence of yeasts in various marine habitats is a well established fact. Little information is yet available however regarding both the quantitative and qualitative distribution of yeasts in these habitats which could help to indicate the major sites of their reproduction or

to/

TABLE 15

Latin designations for English fish names:-

English Names		Latin Designations
Bream	-	Lagodon rhomboides
Cod(Atlantic)	-	Gadus callarias, syn.G.morhua
Dab	-	Pleuronectes(Limanda)ferruginia
Dogfish	-	Squalus acanthias
Gurnard	-	Trigla species
Haddock(Atlantic)	-	Melanogrammus(Gadus)aeglefinus
Hake(Atlantic)	-	Merluccius merluccius
Herring	-	Clupea harengus
Lemon sole	-	Microstomus(Pleuronectes) microcephalus
Ling	-	Molva molva
Mackerel	-	Scomber scombrus
Plaice	-	Pleuronectes platessa
Saithe	-	Pollachius(Gadus) virens
Salmon	-	Salmo salar
Skate	-	Raja species
Turbot	-	Scophthalmus maximus
Whiting	-	Gadus(Odontogadus)merlangus
Witch	-	Glyptocephalus cynoglossus

to elucidate their relationship with fish.

In this study it was not practicable to undertake broad investigations of the yeast flora of the whole marine environment. However attempts have been made to study a few sea-water samples taken from the Clyde Estuary at a location near where several of the "hauls" were made for the First and Second Collections.

In order to take sea-water samples an apparatus modelled on the "J-Z Bacteriological Water Sampler" designed by Zobell(1941), but somewhat simplified, was constructed - see Figures 2 and 3. This consisted of a carrier "H"(in this instance made of wood) weighted with lead sheets "E" and adapted for ready connection to a standard hydrographical wire or cable "A" at "D". A sterilised, partially evacuated glass bottle(in this case a small mineral-water bottle proved quite suitable) "L" is placed in the carrier, fixed by screws "J", and thin glass tubing "C" connected to the bottle through rubber tubing "F" is placed in position as indicated in Figures 2 and 3. When the apparatus is lowered(see Figure 4) to the desired depth a metal messenger is sent down the suspending wire "A" to strike the lever (held on a swivel screw at "G") at "B" thus forcing up the part labelled "M" to strike the glass-tubing and cause it to break at a file mark "FM". The rubber tubing "F" immediately straightens out and the sea-water enters the bottle at a point several inches from the apparatus, thus lessening the chances of contamination of the sample.

There/

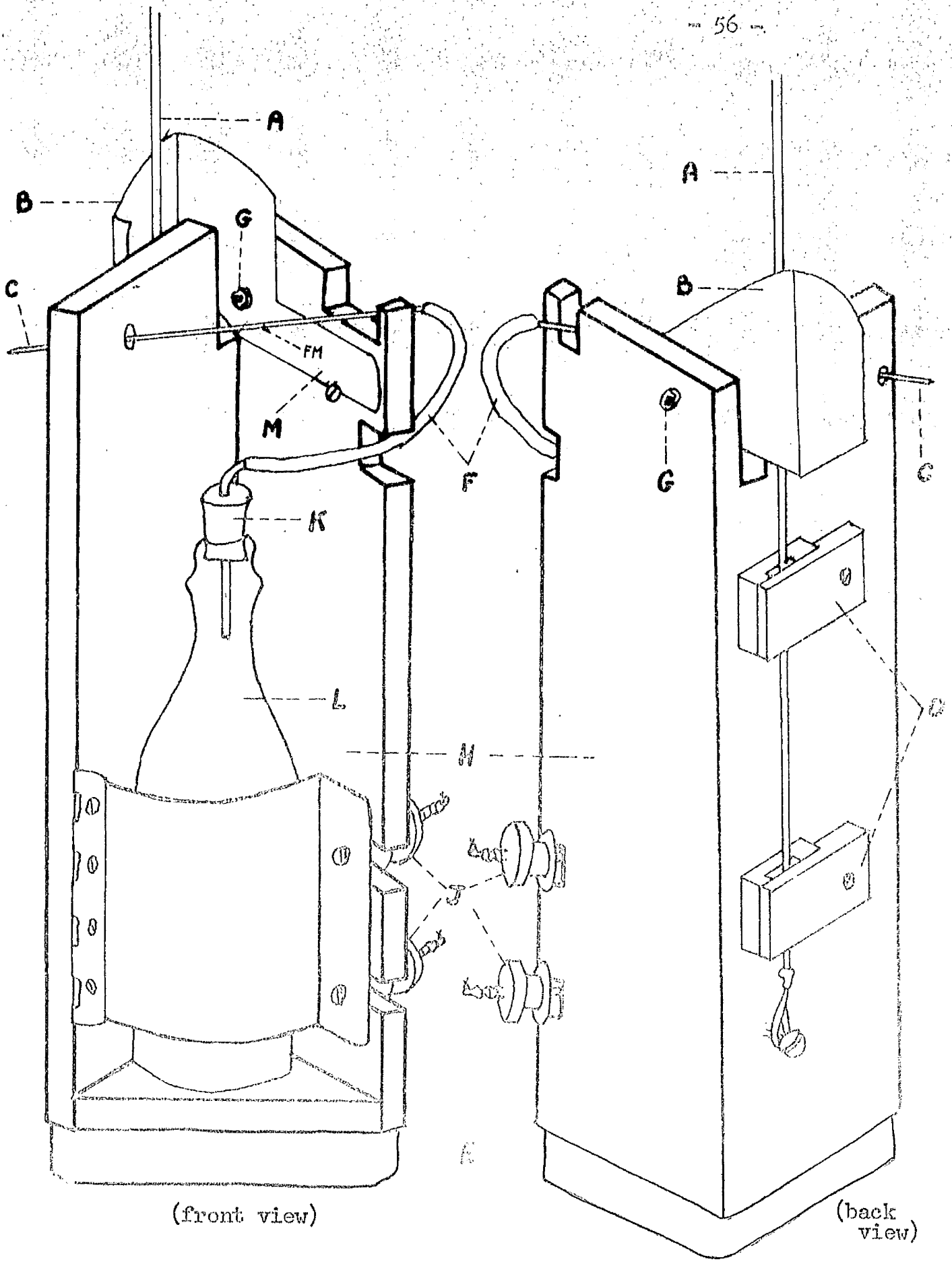


FIGURE 2 Sea-water sampling apparatus.

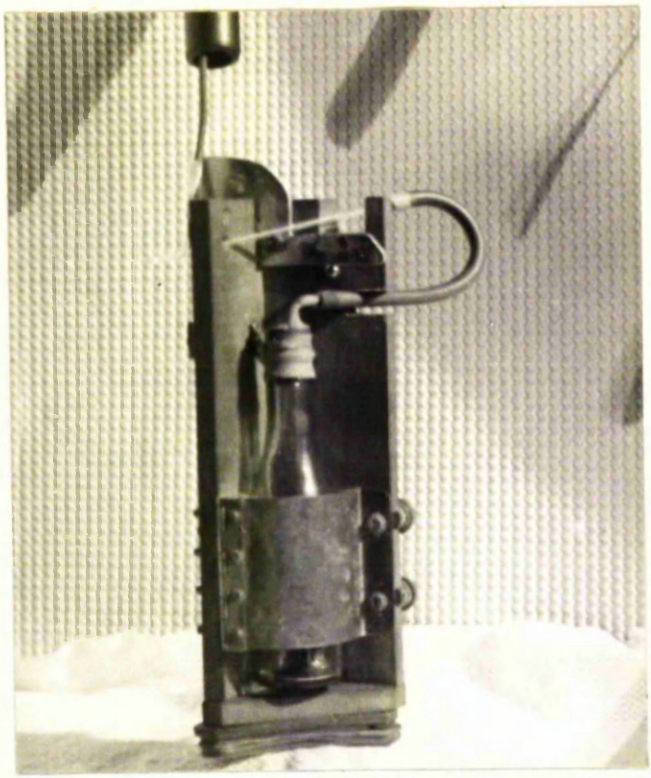


FIGURE 3 Sea-water sampling apparatus

FIGURE 4 Sea-water sampling apparatus being lowered into the sea.

There is little possibility of adventitious organisms entering the bottle while the apparatus is being hauled to the surface as the pressure of the water inside the bottle equals that on the outside and the tendency is for water to be forced out of the bottle. On reaching the surface the rubber tubing was closed tightly with a clip until the broken glass tubing could be sealed with a flame. Before use the sampling bottles were assembled with an air-filter attached to the open end of the glass tubing and autoclaved at 10 lb/square inch pressure for 20 mins. After autoclaving a partial vacuum was created in the bottle by attaching the air-filter to a vacuum line. The bottle was then sealed by collapsing and drawing out the glass tube between the bottle and the air-filter. All the joints between glass and rubber tubing were sealed with waterproof adhesive tape. The file mark "[illegible]" was made just prior to lowering the bottle into the sea.

Samples of sea-water (approximately 100 ml) were taken just off White Bay at the north end of the Great Cumbrae, in the Clyde Estuary, on 28th August 1961. Owing to inclement weather conditions only eight samples were taken; numbers one to seven were taken at 20 - 30 metres and number eight from just below the surface at approximately 0.3 metres.

On reaching the surface the samples were carefully sealed and on arrival at Glasgow four hours later they were immediately placed at 4°C. At no time during their transportation did the atmospheric temperature/

temperature surrounding the samples rise above 25°C. After sixteen hours storage at 4°C the samples were removed for examination and for this purpose it was decided to employ membrane filters. In this connection Kriss(1959) while investigating the micro-flora of the Black Sea found membrane filters facilitated the detection of yeasts to such an extent that this examination technique was adopted with considerable success by him and his co-workers throughout all sub-:sequent studies of the marine yeast flora. The detection of some species antagonism and the extreme smallness of some yeast colonies on the filters did however somewhat hamper isolations and Kriss(1959) suggested that in view of these factors only a broad outline of the quantitative distribution of yeasts in the seas could be indicated by this technique. More recently Fell and van Uden(1961) and van Uden and Castelo-branco(1961) also employed membrane filters while studying sea-water yeast flora.

In this present study "Oxoid" membrane filters - 5 cm. in diam., Grade AP - were employed. Prior to use these were interleaved with filter paper, wrapped singly in grease-proof paper, kept flat with weights, and autoclaved at 15 lbs/square inch pressure for fifteen minutes. It was found previously that sterilisation in situ caused the membrane to curl and hence the filter-holder was sterilised separately, loosely assembled and wrapped in Kraft paper. The sterile membrane was inserted aseptically before each filtration.

The/

The sea-water samples were filtered in two portions of approx-:imately 50 ml. each, a fresh, sterile membrane being employed for each filtration. The membrane from one filtration was placed with the effluent-side in contact with solid "MYGPS" at pH 5.0 contained in petri-dishes, while the other was immersed in 10 ml. broths of "MYGPS" at pH 5.0. These cultures were incubated at 25°C except for those from sample number two which were placed at 4°C.

After four days on the "MYGPS" plates all the membranes(except those from sample 2) were found to be covered with bacteria and yeasts to such an extent that no distinctive colonies were obvious. Sections of this growth were therefore scraped off and placed in broths of "MYGPS" at pH 5.0. After two days, examination of these broths and those containing filters revealed in eight of the fourteen cultures the presence of yeasts and samples from these were immediately seeded onto plates of solid "MYGPS" at pH 5.0. The remaining broths were subcultured into fresh "MYGPS" broths at pH 4.0 and 3.5 respectively which were then examined at intervals and plated out if yeast growth was apparent. All the cultures obtained were purified by replating and taking a single colony for a streak culture.

The two filters used for sample two were removed from 4°C after three weeks and were found to contain growth of yeasts and bacteria to the same extent as that found on those from the other samples incubated at/

at 25°C for four days. Sections from the membrane on the plate and a sample from the broth containing the membrane were placed in "MYGPS" broths at pH 4.0 and 3.5 respectively and pure yeast cultures obtained as before.

Eleven pure cultures of yeast were obtained (see Table 16).

TABLE 16

Cultures from sea-water samples, White Bay, Great Cumbrae, Clyde Estuary, 28th August 1961:-

Sample No.	Depth	Cultures obtained from these samples
1	20-30 metres	No.501
2	"	No.510, No.511
3	"	----------
4	"	No.504
5	"	No.502, No.503
6	"	No.505, No.506
7	"	No.507
8	approx. 0.3 metres	No.508, No.509

Again single colonies representing one type on a plate were taken. Only one type of yeast colony was found on each plate and inhibition of yeast growth may have occurred due to the large numbers of bacteria which also developed on the filters. It is suggested that a lower pH e.g. 4.0 and the inclusion of antibiotics in some of the isolation media may help to exclude bacteria which are met with in great numbers in estuarine and off-shore waters.

Conclusion

Altogether from various marine sources two hundred and thirty-five yeast cultures have been collected and of these one hundred and eighty-nine were isolated from marine fish during this survey. With each fish sampling expedition improvements in the sampling and yeast isolation methods were reflected in the increasing numbers of positive samples obtained. The inclusion of an antibiotic mixture - (10 mg/l aureomycin, 2 mg/l chloramphenicol, 2 mg streptomycin sulphate) in the isolation media was a further improvement which allowed more types of yeast to develop as well as precluding bacterial growth. It must be borne in mind however that although the methods used in this survey have allowed the isolation of a reasonable number of yeasts for study further investigations employing a wider range of media and methods may be necessary to ensure the isolation of the complete spectrum of yeast species present in this marine environment.

MAINTENANCE OF CULTURES

Maintenance Media

The collection has been maintained as streak cultures on "MYGPS" medium at pH 5.0 held at 4°C in one-ounce screw-cap bottles. Sub-cultures are made every eight to ten weeks and only a few strains, i.e., six, three of which were of the genus Rhodotorula, have failed to survive. In the initial stages of making the collections it was of course not known to what extent the organisms would survive this type of maintenance involving relatively frequent subculturing. Hence it was decided to investigate the value of freeze-drying (lyophilisation) when applied to these isolates.

Freeze-Drying

Introduction:

This technique has been used extensively for the preservation of bacteria and moulds but in view of the relatively scant information regarding its use for yeasts, freeze-drying is used mainly as an ancillary method for preservation of the latter organisms by most workers. Freeze-dried yeast cultures have been found to remain viable for considerable lengths of time, e.g., Haynes et al. (1955) reported that many of the six thousand yeast strains in the collection of the Northern Regional Research Laboratory at Peoria which had been freeze-dried/

freeze-dried were still viable after ten years' storage.

Only a few other reports concerning the freeze-drying of yeasts are available. Wickerham and Andreason (1942) found 95.1% of the three hundred and eighty-four yeast cultures freeze-dried by them were still viable after twelve months, whereas only 80% of the same cultures maintained as stab cultures survived over this period. Kirsop (1955) freeze-dried eighty-three strains of yeast representing twelve different genera and only two of these, a strain of Candida and a strain of Brettanyomyces, failed to survive after nine months. Many of the other strains, however, notably those of the genus Saccharomyces, exhibited a very low percentage viability, i.e., below 1.0%, after nine months' storage. Atkin et al. (1949) also found a low percentage viability after freeze-drying cells of Saccharomyces cerevisiae, only 0.2% of the cells of the original suspension remaining alive.

Method:

The method of freeze-drying adopted for this test was that which has been developed for routine use in this laboratory for bacteria. A different freeze-drying fluid was, however, employed for the yeasts which comprised one part "MYGP" containing 30% glucose (filter-sterilised) and three parts sterile horse-serum. The inclusion of glucose in the freeze-drying fluid has been found to reduce cell mortality and Fry and Greaves (1951), working with bacteria, suggested that this may be due to some regulation of the final moisture content of the cultures by the glucose. The horse-serum is used here as a protective/

protective colloid.

Brady (1959) noted that mature cultures are better able to survive freeze-drying than young ones. Accordingly, the inocula were taken from forty-eight-hour cultures grown on "MYGP" slopes by adding 2.0 ml of the freeze-drying fluid, mixing thoroughly and transferring 0.25 ml suspension to the bottom of six appropriately labelled sterile tubes (approximately 10 cm long and 0.5 cm in diameter) covered by lint hoods. The tubes and their contents were then subjected to the primary drying process by centrifuging five minutes under vacuum; P_2O_5 contained in separate trays was used as a drying agent. After centrifugation the cultures were retained in the centrifuge carrier under vacuum until the pressure reached 9-10μ of mercury. The air outlet valve was then opened, the vacuum-pump stopped and the tubes removed. The lint hoods were then replaced aseptically by sterile cottonwool plugs which were placed well down the tubes, a few crystals of freshly heated indicator silica gel (blue) added and the tubes constricted in a flame to produce a narrow neck just above the silica gel.

The tubes were then placed on a manifold attached to the centrifuge-carrier inside which was a tray containing P_2O_5. A vacuum, equivalent to 10μ of mercury, was produced and maintained for approximately sixteen hours when the tubes were removed by sealing in a flame while still under vacuum.

Viability counts:

A/

A modified version of the count described by Miles and Misra (1938) was used to determine the viability of the cultures immediately before and after freeze-drying and again approximately two years later. For the initial count a one-in-ten dilution in Ringer's fluid was made with 0.25 ml of the suspension in freeze-dried fluid. Further serial dilutions one-in-ten were prepared and 0.02 ml of each dilution were placed in five different sites on plates containing "MYGP" medium which had been dried to prevent the drops spreading. After about forty-eight hours counts of the average number of colonies per spot, developed on a plate from a certain dilution, were made and the number of cells present in the original suspension calculated. Counts were made of the freeze-dried cultures in a similar fashion by reconsti-:tuting the contents with Ringer's fluid to a volume of 2.5 ml from which one-in-ten dilutions were made, as before.

Fourteen strains were used for the test as indicated in Table 17. The collection of these strains has been described in the previous section under the heading "Miscellaneous".

Results:

The percentage viability of each yeast strain, two days and two years after freeze-drying is given in Table 17. It can be seen that there is considerable initial loss of viability; the average figure for the viability of all the strains two days after freeze-drying was 52.6%. This is a considerably higher average figure than that obtained/

TABLE 17

Survival of Freeze-dried Cultures:-

Strain No.	Species	Number of Cells inoculated into ampoules	% Viability 2 days later	% Viability 2 years later
281	D.kloeckeri	175×10^6	50.0	9.8
431	"	106.5×10^6	63.0	39.6
11	"	130×10^6	40.3	13.1
54	"	180×10^6	73.0	54.1
Y3	"	207.5×10^6	85.5	33.8
254	Rh.glutinis	207×10^6	52.3	30.0
40red	"	14×10^6	95.0	42.9
12pink	Rh.mucilaginosa	150×10^6	32.1	30.1
T4	C.parapsilosis	190×10^6	34.2	10.9
T6	"	157.5×10^6	94.1	31.1
T7	"	205×10^6	90.1	55.2
Light I	"	38×10^6	1.1	1.1
Light II	"	28×10^6	1.6	1.5
845	T.famata	94×10^6	25.0	4.5
		Average -	52.6	25.9

obtained by Kirsop (1955), i.e., 8.6%. However, it should be noted that she included in her investigation many strains of the genus Saccharomyces which proved very sensitive to the freeze-drying process. On the other hand, she found that strains of the genera Debaryomyces, Shizosaccharomyces, Torulopsis, Candida and Rhodotorula survived in the greatest numbers, the average percentage viability of each genus being - 42.5, 27.3, 16.2, 10.6 and 10.1, respectively. These figures obtained by Kirsop (1955) are somewhat lower than those obtained in the present test, i.e., Debaryomyces - 62.3%, Rhodotorula - 59.8%, Candida - 44.2%, Torulopsis - 25.0%. Brady (1959) also found strains of Torulopsis, Debaryomyces, Candida and Rhodotorula to be amongst the genera best capable of surviving freeze-drying.

After two years' storage the average percentage viability dropped by a further quarter to 25.9%. Kirsop (1955) also noted a lesser drop in viability during a storage period of nine months than that incurred initially as a result of freeze-drying.

Conclusion:

The satisfactory figures in terms of survival of the cells, except for the two strains Light I and Light II, indicate that freeze-drying is potentially a useful method for preservation of these strains of marine origin. It should be borne in mind, however, that some workers have reported changes in morphology and physiology of freeze-dried yeast cultures. Atkin et al. (1949), for example, found freeze-dried/

freeze-dried Saccharomyces cerevisiae cells to be more exacting in their vitamin requirements, whereas Kirsop (1955) found evidence to the contrary with her strains which were found to be less exacting in this respect. She also found no other physiological or morpho-:logical changes in the cultures, but Subramaniam and Prahlada Rao (1951) have reported some changes in giant cell characteristics of freeze-dried cultures. Haynes et al. (1955) state that they found no change produced by freeze-drying in the major classification characteristics of the yeasts in their collection and they consider that this method of preservation may allow the yeasts to retain those properties which sometimes prove unstable on prolonged cultivation.

IDENTIFICATION OF ISOLATES

Introduction

Probably the most comprehensive and readily available classification, encompassing both sporing and non-sporing yeasts, is that of Lodder and Kreger-van Rij (1952) which evolved from the work of the Delft school, i.e., Stelling-Dekker (1931), Lodder (1934), Diddens and Lodder (1942), under the influence of Kluyver. This system is, however, essentially a practical one and therefore, as is to be expected, certain anomalies have been found to exist (Barnett, 1960; Santa-maria, 1960; Kreger-van Rij, 1962), often making identification of fresh isolates or designation of new species and varieties difficult.

Methods for the evolution of a more genetically based classification system in which the importance attached to morphological criteria is reduced in favour of the biochemical were proposed by Wickerham (1951). In a comprehensive study of the genus Hansenula employing more numerous and extensive biochemical tests than Lodder and Kreger-van Rij(1952), a possible phylogenetic relationship amongst the different species was indicated. A monograph dealing with yeast taxonomy prepared by Kudriavtzev(1954) is a similar attempt to make a more natural classification system in which nutritional behaviour is considered more valuable than morphological appearance. At the time of undertaking this investigation, however, this monograph was available only in Russian and had received but scant attention in many countries. Zsolt (1959) advocated a broader approach to yeast taxonomy based on phylogeny requiring the isolation of many new strains and comparative studies/

studies of these with older cultures in order to detect the manner in which their individual characteristics have come into existence, particularly those concerning the fermentation and assimilation of a large number of carbon compounds. A new system proposed by Novak and Zsolt (1961) although embodying a large number of well-defined categories which might facilitate the classification of new genera and species is still a somewhat rigid system lacking phylogenetic basis.

Barnett (1960) proposed that yeasts could be classified mainly on biochemical tests to reveal the presence or absence of particular single enzymes. This proposal, however, was strongly opposed by Roberts and Thorne (1960) who pointed out that a classification of this nature ignoring such features as morphology, spore-forming ability or alternation of generations is merely cataloguing and, while it may be extremely useful in improving knowledge of the biochemical potentialities of many kinds of yeasts, the primary concern of any microbial classification should be an attempt to demonstrate natural relationships of the organisms involved. These latter workers believe that the Dutch classification system will not be superseded in the future but rather revised on a more genetical basis, emcompassing some alternative views of yeast classification such as those cited above and containing in addition more details of sexual processes, of life cycles, of comparative cytology, of morphological responses to changing environmental conditions and of natural habitats and distribution/

distribution as well as of biochemical activities.

In a recent paper Kreger-van Rij (1962) has emphasised the important part played by biochemical criteria in yeast taxonomy and states that a study of more properties and strains leads to an improved knowledge of valuation and properties within one species. She suggests, however, that the value of biochemical criteria alone for characterising higher taxa, i.e., genera, is still a matter for discussion and points out that the classification of asporogenous yeasts put forward in the Dutch system which uses biochemical criteria without the guidance of important morphological characteristics is not satisfactory as a natural system.

Bearing the above considerations in mind, it was decided to use the system of classification proposed by Lodder and Kreger-van Rij (1952) because of its wide general acceptance. Further tests, most of which were suggested by Wickerham (1951), have also been carried out in order to provide more information which not only could be of immediate use in revealing the potentialities of individual strains and show any variation within each species group, but could also help in placing the strains in future classification systems which might require a wide knowledge of the yeasts' various characteristics along the lines indicated by Roberts and Thorne (1960). The methods used for these latter tests and the results obtained from them will be discussed under the section "Supplementary Tests".

Methods

Some/

Some slight modifications of the techniques and materials employed by Lodder and Kreger-van Rij (1952) have been made in this study and these will now be discussed:-

Yeast Cell Measurements:

Cell dimensions obtained by standard procedures have been found to be a stable characteristic of yeast strains (Morris, personal communication) and is, therefore, an important criterion in yeast classification. The methods adopted in this study are modelled on those of Lodder and Kreger-van Rij (1952) who measured at least twenty cells from two-to-three-days old malt wort and malt agar cultures incubated at 25°C. In this study cells were taken from three-days old cultures grown in the latter media at 25°C and employing a projection microscope a scaled image of the cells (x 2,000) was thrown onto a screen. One hundred cells were then traced and afterwards their average dimensions and range of cell size determined. These values for each strain are given in the Appendix.

In their monograph Lodder and Kreger-van Rij (1952) give the value of the smallest and largest cells observed in a limited field. Such information gives no indication of how the dimensions of the other cells fall between these limits. In calculating the average dimensions of a relatively large number of cells it was hoped to obtain a value which is less influenced by biological variation and choice of field and hence might prove a more useful criterion for classication purposes./

purposes.

It is obvious that this method is tedious and laborious, especially when large numbers of cultures are being studied concurrently. The use of an electronic particle counter was, therefore, considered in this connection. Such instruments have been used successfully for routine size measurements of small cells such as bacteria and spores (Kubitschek, 1958). Unfortunately a few experiments carried out in this laboratory indicated that this method can only estimate the volume of the cells and gives no indication of their shape.

Spore Formation:

Except in the case of certain Saccharomyces species the factors governing spore formation in yeasts are little known. However, it is commonly believed that yeasts should be cultivated in a fully nutrient medium, i.e., presporulation medium, prior to transference to the spore-inducing media. Accordingly, all the strains under investigation were first grown in V8 broth (Wickerham et al, 1946) for three days at 25°C on two successive occasions.

The fact that certain yeasts appear to respond differently on various sporulation media and the lack of understanding of this phenomenon made it necessary to employ a wide range of media such as Goradkova's agar, Glucose-sodium acetate agar (Adams, 1950), cement blocks (Hartelius and Ditlevson, 1953), carrot and potato plugs, McKelvey's/

McKelvey's carrot infusion medium and V8 agar.

As mentioned by Lodder and Kreger-van Rij (1952) loss of spore-forming ability in yeast strains can result from a period of laboratory cultivation and therefore in this study care was taken to set up cultures for the demonstration of spores as soon as possible after pure cultures were obtained.

Assimilation Tests:

Initially these tests were made using cells from a three-day old streak culture on "MYGP" agar grown at 25°C which were washed twice aseptically with sterile distilled water using centrifugation. The resulting cell suspension was then divided in two, half being placed in another sterile bottle and after a further centrifugation of the two suspensions, the supernatant being poured off aseptically, five ml sterile blank nitrate assimilation medium (Lodder and Kreger-van Rij, 1952) was added to one bottle and five ml sterile blank carbon assimilation medium to the other. Both bottles and their contents were then incubated at 25°C for two days. Such a period of "starvation" on blank media prior to testing assimilation reactions was recommended by Barnett and Ingram (1955) as a means of exhausting any intra-cellular stores of carbon or nitrogen capable of being utilised by the cells and thus interfering with the tests. Liquid cultures were then inoculated from the appropriate "starvation" culture.

Later/

Later, in view of the large number of yeast strains to be tested it was decided to use auxanographic methods employing the replica plating technique for the sugar tests. Replica plating was developed by Lederberg and Lederberg (1952) for the detection of biochemical mutants in bacteria and was subsequently applied successfully in the study of carbon assimilations by yeasts by Shifrine et al.(1954). In this study a multi-inoculator similar to the one described by Beech et al.(1955) was employed instead of the velveteen block of the former workers, thus dispensing with an initial inoculation plate and the risk of nutrient carry-over.

A loopful of the two-day starved cell suspension in blank carbon assimilation medium was placed in a bottle containing five ml sterile water, and, after shaking, one ml of this dilute suspension was trans-:ferred aseptically to a small sterile tube (3.5 cm long and 0.8 cm in diameter) which was placed in position in the tube-holder and covered by a sterile metal petri-dish lid. A maximum of twenty-three such tubes can be accommodated in the holder. When all the tubes contain-:ing inocula were in position the lid over the tubes was removed and the sterile inoculator placed in position in the tube-holder long enough only to allow its metal prongs to dip into the yeast suspensions. The inoculator was then transferred to the plate-holder where the metal prongs covered with inocula were allowed to impinge lightly on the surface of a test medium held in a petri-dish. The inoculator was then removed from the plate-holder, the lid of the plate replaced and another/

another plate put in position. A range of test media was thus inoculated, working as quickly as possible to avoid aerial contamination. Dripping during inoculations was lessened by the inclusion of one drop of a sterile solution of Tween 80 (1.0%) in the dilute yeast suspension used for the inoculum.

The test medium on each plate consisted of 13.5 ml basal carbon assimilation medium (Lodder and Kreger-van Rij, 1952) containing washed agar mixed with 1.5 ml of a filter sterilised 10% w/v solution of a single sugar (glucose, maltose, galactose, lactose or sucrose) and two drops of a concentrated vitamin solution (Lodder and Kreger-van Rij, 1952) also previously sterilised by filtration. A blank plate containing another 1.5 ml basal carbon assimilation medium instead of sugar solution was also prepared.

After inoculation the plates were held at 25°C and the results read after three days and one week. A few yeasts were found to grow rather poorly on these plates and their growth was increased by using more concentrated inocula. Generally however, good, clear results were obtained using this method. Interaction between colonies growing on sucrose plates due to the diffusion of hexose sugars after sucrose hydrolysis was reported by Shifrine et al (1954) but no such interaction was found in this present study. The absence of the latter phenomenon was inferred from the fact that no matter what combination of yeast strains was included on each test plate, reproduceable/

reproduceable results were obtained for each yeast.

Splitting of arbutin:

This test was carried out according to the methods of Lodder and Kreger-van Rij (1952). Initially arbutin obtained from L. Light & Company was employed. By the use of known positive strains it was found that this compound produced a rather insensitive medium. A repetition of the tests using B.D.H. arbutin produced an altogether more sensitive medium as indicated by the stronger positives produced and the detection of hydrolysis by strains, e.g., Torulopsis inconspicua (var), which appeared negative on the former medium.

Results

In the Appendix all the strains comprising each species are listed, as are groupings of strains within the species which have been made according to the various results obtained in the routine and supplementary tests (see page 99). Differences between the strains in each species group and that of the type species are also indicated.

A) Debaryomyces kloeckeri:

The ninety-eight isolates given this designation resembled closely the description of the type species. Approximately 65% of these isolates were able to ferment glucose and sucrose (weakly) or glucose only (in some cases weakly) and the rest were unfermentative. Variation amongst the isolates was also observed with regard to their ability/

ability to split arbutin, i.e., approximately 9% lacked this ability.

Using the ability to ferment sugar(s) and to split arbutin as criteria, the isolates can be placed in three divisions - those possessing both these abilities, those able to split arbutin but unable to ferment sugar(s) and those unable to split arbutin or to ferment sugar(s). These divisions are sub-divided in the Appendix according to information gained from the supplementary tests. Thus, the first division comprises Groups 1 to 4; the second division comprises Groups 5 to 7 and the third division comprises Groups 8 and 9.

Little difference is apparent amongst the scatter of average values of the cell dimensions of the strains comprising the major divisions of this species (see Fig. 5) and it seems, therefore, that if these divisions comprise different strain types the dimensional values show no indication of it. It is interesting to note that although these measurements show no correlation with the above grouping there is a close correlation of the ratio of the dimensions of the long-to-short axis for most strains.

B) Debaryomyces subglobosus:

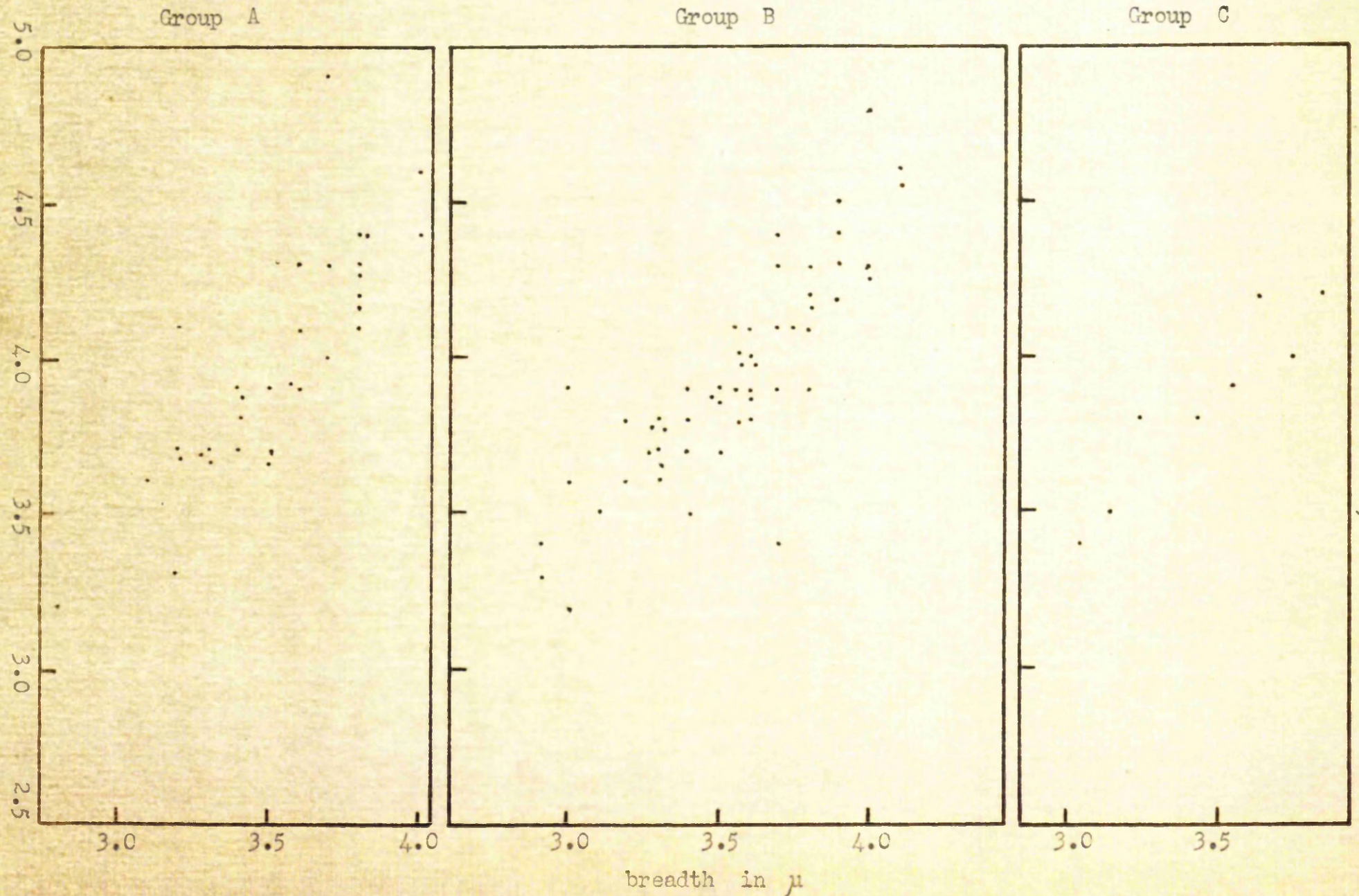
The nine isolates given this designation resembled closely the type species, except that four were unable to ferment glucose. This property is a characteristic of the type species.

C) Torulopsis inconspicua:

These/

FIGURE 5

Average dimensions of individual isolates of D.kloeckeri after 3 days' growth in malt wort at 25°C:-



These four isolates resembled closely the description of the type species, except that two were able to split arbutin weakly. This ability is not possessed by the type species.

D) Torulopsis inconspicua (var):

The twenty-four isolates given this designation differed from the description of the type species Torulopsis inconspicua by their ability to assimilate potassium nitrate and the fact that many were able to split arbutin weakly.

Recently several new species of Torulopsis, resembling very closely these strains from fish have been described, i.e. T.norwegia nov. species (Reiersöl, 1958) from the sputum of a patient suffering from tuberculosis, T.saccharum nov. species (Tabey Shehata, 1960) from sugar cane washing water, T. domercquii nov. species (Van der Walt and Van Kerken, 1960) from vatted wine, and T. vanzylii nov. species (Van der Walt and Van Kerken, 1961) from mould growth on the floor of a refrigerated wine cellar.

A further indication that these types of yeast are more common than had been previously supposed is the fact that isolations of nitrate positive strains of Torulopsis from processed poultry have been reported by Walker and Ayres (1959); and Reiersöl (1958) stated that Wickerham had in his possession a few yeast strains resembling T. norwegia nov. species.

The/

TABLE 18

Comparison of Torulopsis inconspicua(var) and of new species of Torulopsis capable of assimilating nitrate.

SPECIES	T.domercquii (Van der Walt & Van Kerken,1960)	T.saccharum (Tabey Shehata,1960)	T.vanzylii (Van der Walt & Van Kerken,1961)	T.norwegia (Reiersol,1958)	T.inconspicua(var)
MALT WORT					
Shape	round-long oval	spherical-short oval	round-short oval	round-short oval	oval-round
Size in u	(1.5-3.0)x(2-4)	(1.8-3.6)x(1.8-4.5)	(2.4-6)x(2.7-6.6)	(2.4-5.2)x(2.8-5.8)	average(4x4.4)
1 mo.at 17oC	ring and sediment	ring and sediment	ring and sediment	ring and sediment	sediment only
MALT WORT AGAR					
Shape	as in malt wort	------	as in malt wort	as in malt wort	as in malt wort
Size in u	(1.5-3)x(2-4)	------	(2.4-8)x(2.4-5.4)	(2.3-5.2)x(2.6-6)	average(3.5x3.8)
1 mo.at 17oC	brownish-cream, glistening,flat.	white-cream,smooth, glistening	greyish-white,flat, smooth,glistening.	cream,smooth, glistening	greyish-white, smooth,glistening
SUGAR FERMENTATION	nil	nil	glucose(weakly)	nil	glucose(weakly) or nil
CARBON ASSIMILATION	glucose and ethanol only	glucose and ethanol only	glucose and ethanol only	glucose and ethanol only	glucose and ethanol only
NITRATE ASSIMILATION	+ve	+ve	+ve	+ve	+ve
ARBUTIN SPLITTING	-ve	-ve	+ve	+ve	weak +ve or -ve

The descriptions of these new Torulopsis species together with those of the isolates classified as Torulopsis inconspicua (var) in in this present study are set out in Table 18. On comparing the former strains it is obvious that the absence of any major differences amongst them makes it extremely likely that they will eventually be grouped into one Torulopsis species, perhaps T. norvegia, but in view of the existing taxonomical confusion the provisional diagnosis of T. inconspicua (var) has been made for these fish isolates.

On the basis of arbutin hydrolysis and glucose fermentation these marine isolates can be placed in four divisions which comprise Groups 1; 2 and 3: 4 and 5 and 6 to 9, respectively. These are listed in the Appendix. No other characteristics, including those of cell measurements, appear specific for any of the four divisions.

Several species of Torulopsis have been found to be haploid strains of heterothallic yeasts, e.g., T. molischiana is a haploid strain of Saccharomyces lactis (Wickerham and Burton, 1952). It was hence considered necessary to investigate these marine strains for the possible existence of mating types. Cultures in V8 broth were mixed in batches of four and sporing media inoculated by pairs of these batches in every possible combination so that each strain had contact with each other. No sporing was, however, observed in any culture during six weeks' incubation at 25°C and, therefore, the existence of mating types in these strains was considered unlikely.

E) Torulopsis candida:

The/

The five isolates given this designation resembled closely the description of the type species, except that two were unable to split arbutin. No mating types were found amongst them and their average cell dimensions fell within close limits characteristic of the type species.

F) Torulopsis famata:

These five isolates resembled closely the type species. Only three were able to ferment glucose and did so weakly. This ability to ferment glucose is a variable characteristic of the type species. No mating types were found amongst the isolates and their average cell dimensions fell within close limits characteristic of the type species.

G) Torulopsis pseudaria (Zsolt, 1958):

The description of this new yeast species isolated from vineyard soil in Hungary by Zsolt (1958) is set out in the Appendix. Strains 43/6 and 402, although given this designation, did exhibit some differences from the authentic description, i.e. in cell shape after three days' growth at 25°C in malt wort and on malt wort agar; in appearance of growth on the latter media after one month at 17/20°C and in assimilation of ethanol. As for the other Torulopsis species, no mating types were found.

H) Candida parapsilosis:

Some initial difficulty was experienced in identifying the twenty-five isolates now classified as C. parapsilosis. This arose from/

from the difficulty of interpreting the descriptions provided by Lodder and Kreger-van Rij (1952). That is, according to the key of the genus Candida, C.scottii should either fail to ferment sugar or give only a weak reaction, whereas the description of this species excludes fermentation of sugars. Concerning these isolates, fermenta-:tion was absent in approximately one-sixth of them and very weak fermentation of glucose, and in some cases of galactose, occurred in the rest. The morphology of the pseudomycelia of the various strains varied between that described for C.parapsilosis and C.scottii. The deciding factors in classifying these isolates as C.parapsilosis was their inability to assimilate nitrate and their general agreement morphologically with this type species. However, no giant cells were observed in the pseudomycelia and the streak cultures, after one month's growth at 17/20°C, appeared white rather than cream-coloured to yellowish. Eighteen of the isolates also differed from the type species by being able to split arbutin and this property, together with the absence of giant cells in the pseudomycelia, is characteristic of C.parapsilosis var. intermedia. However, their ability to produce good growth in ethanol and the absence of a dull, thin pellicle in malt wort cultures incubated for two weeks at 17/20°C are not charac-:teristic of the latter variety and it was decided to classify them as C.parapsilosis.

In view of the fact that several strains either failed to show any sugar fermentation or did so only weakly it is of interest to note that/

that Roth et al (1962) found that recently isolated strains of this species from deep seas also failed to ferment sugars. A period of growth, however, on an enriched substrate allowed the latter strains to acquire fermentative ability which could be lost again after a period of starvation in sterile water. No changes in oxidative ability were found during these experiments and Roth et al (1962) suggested that the fermentative capacity of C. parapsilosis declines in an environment having a lower concentration of organic compounds such as is found in open oceanic waters.

On the basis of sugar fermentation and arbutin hydrolysis the isolates can be placed in six divisions which comprise Groups 1; 2; 3 and 4; 5 to 15; 16 and 17; 18 and 19, respectively, as indicated in the Appendix. The cell dimensions of the different strains within each of these six divisions appeared to vary too much to be of any characteristic value.

Except in their smaller cell dimensions, their ability to form well developed pseudomycelia and their inability to form spores, these strains also resemble Metschnikowia zobellii (vanUden and Castelo-branco, 1961) and the probability that these isolates may be haploid strains of this species could not be overlooked. Mating experiments were, therefore, performed as for the Torulopsis species. After six weeks' incubation no cell conjugation or spore formation was observed, hence it was concluded that no mating types exist

amongst/

amongst the isolates.

I) Candida lipolytica (var):

The ten isolates given this designation differed from the type species C.lipolytica in several respects, the chief of which was their ability to assimilate nitrate. Other differences were the absence of true mycelia, the appearance of growth in malt wort and on malt wort agar after one month at 17/20°C, good growth with ethanol as sole carbon source and the occurrence of cylindrical cells. Notable exceptions in the possession of the last-named characteristic were strains Her 3 and Her 7.

Differences amongst the isolates in their ability to split arbutin, a variable characteristic of the type species, were observed, i.e., two were unable to split arbutin, two did so slightly and the others strongly. Although differences in cell dimensions and morphological appearance were apparent between isolates (Her 3,Her 7) and the others it is interesting to note their general similarity, bearing in mind that the former were isolated from spoiling smoked herring and the latter from newly caught fish in the North Sea. Mating experiments with these isolates failed to demonstrate any mating types.

J) Candida zeylanoides:

Strain 249 resembled the description of the type species except that after three days' growth in malt wort and on malt wort agar the cells appeared somewhat smaller and good growth was observed with ethanol as the sole source of carbon. 'Mycocandida' type pseudomycelia/

pseudomycelia were produced and the isolate split fat.

K) Candida species:

The six yeasts grouped under this heading are those which have the characteristics of the genus Candida but which have been difficult to assign to definite species.

Strain 417s resembled the isolates classified as C.parapsilosis except that it was capable of assimilating nitrate. This latter ability is a characteristic of C.scottii but the isolate was not assigned to this species in view of its much smaller cell dimensions and its inability to peptonise milk, an alkaline reaction being produced instead.

The other five strains are fully described as a group in the Appendix and resemble each other in every respect except that of the pattern of sugar assimilation. However, sugar assimilation is an important classification criterion of the key to the genus Candida (Lodder and Kreger-van Rij, 1952) and therefore, an attempt was made to compare the isolates with type species.

C.scottii is the only unfermentative species having an assimila-:tion pattern similar to strain 419H. This species differs from 419H, however, in having larger cell dimensions, a paler-coloured streak on malt wort agar and inability to split fat or liquefy gelatin. C.melinii has most properties in common with strain 420H, 421m/

421m and 424H but differs from them in its smaller cell size, paler-coloured streak on malt wort agar, its inability to assimilate ethanol, split fat or liquefy gelatin and its ability to split arbutin. No descriptions of Candida species which are unfermentative and assimilate only glucose and sucrose are available for comparison with strain 427s.

The difficulties of comparing these five isolates with type species are therefore obvious. In view of this and the fact that the isolates are of similar ecological origin and resemble each other closely, apart from their sugar assimilation patterns, it was decided to keep them together as a group under Candida species. A system based on more phylogenetic lines might well show these to belong to a sequence in a closely related group.

L) Rhodotorula glutinis var. rubescens:

The fifteen isolates given this designation resembled closely the description of the type species. Four of the isolates were unable to split arbutin, a variable characteristic of the type species. Once again wide differences in cell dimensions were observed.

M) Rhodotorula mucilaginosa:

The eight isolates given this designation resembled closely the description of the type species except strain 1111, which was unable/

unable to split arbutin. A few strains exhibited only weak growth with galactose and some produced a delicate creeping flim on malt wort after one month's growth at 25°C. Some difference was observed amongst the isolates with regard to their cell dimensions.

N) Rhodotorula rubra:

The three isolates given this designation resembled the type species in most respects. Notable differences were the absence of primitive pseudomycelia, weak assimilation of galactose and smaller cell dimensions.

O) Rhodotorula minuta:

The single isolate given this designation resembled the type species except in the appearance of its growth on malt wort agar and in malt wort after one month at 17/20°C. Its cells were also larger and more cylindrical than those of the type species.

P) Pichia membranaefaciens:

The two isolates resembled closely the description of the type species. Weak fermentation of glucose occurred, only one spore per ascus was observed and good growth with ethanol as sole carbon source was obtained.

Q) Trichosporon pullulans:

The single isolate resembled closely the type species in every respect.

R)/

R) Trichosporon cutaneum var. multisporum:

The two isolates resembled closely the authentic description of the type variety. Good growth was exhibited with ethanol as sole carbon source and arbutin was split - both variable characteris-:tics of the type variety.

S) Cryptococcus albidus:

The single isolate appeared to resemble the type species in every respect and could be placed in Group I of the latter according to its small cell dimensions. No growth occurred with ethanol as sole carbon source and splitting of arbutin was weak - both variable characteristics of the type species.

T) Cryptococcus laurentii:

The single isolate resembled the type species closely except that after one month's growth at 17/20°C no ring was formed but a thin creeping film was present. Growth occurred with ethanol as sole carbon source and arbutin was split - both variable characteri-:stics of the type species. Only slow growth was exhibited by this isolate at 25°C and thus diagnostic tests were carried out at room temperature, i.e. 17/20°C.

U) Cryptococcus diffluens:

These two isolates differed in some respects from the type species. These differences were manifested in the appearance of the culture after one month at 17/20°C in malt wort and in galactose assimilation/

assimilation. One isolate, 407, also differed from the type species by its ability to grow with ethanol as sole carbon source. Both isolates were able to split arbutin strongly - a variable characteristic of the type species.

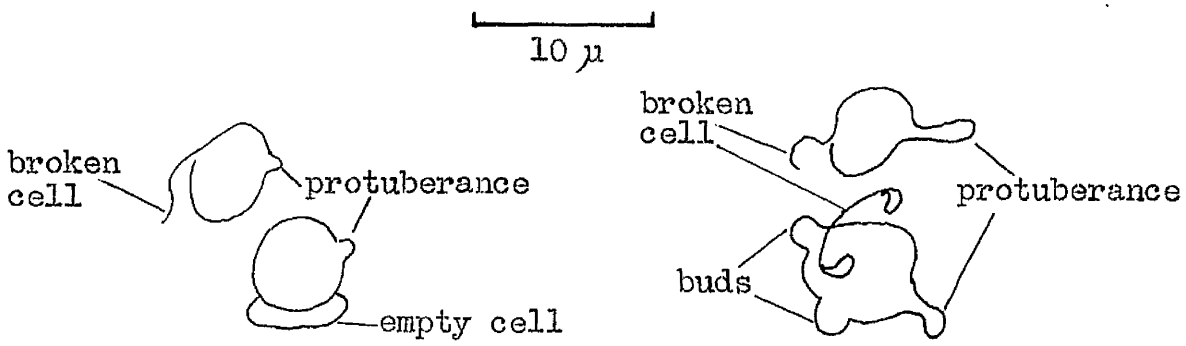
V) Metschnikowia krissii: (vanUden and Castelo-branco, 1961)

The five isolates given this designation resembled the type species except that they were able to ferment glucose weakly and did not assimilate ethanol. The dimensions of the cells of the isolates were on the small side of those given for the type species.

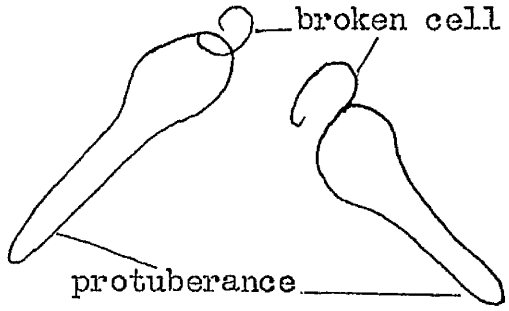
The spores and asci formed on V8 agar andcarrot plugs resembled those described for the type species. Observation of the cells of isolate 505 on carrot plug prior to spore formation revealed broken and empty cells attached to cells which were enlarging to form the typical club-shaped asci and drawings were made of these forms - see Fig. 6. The latter observations seem to suggest that cell conjugation of some form or another occurs prior to spore formation. Attempts, however, to produce these forms again for photographing have so far failed.

W) Pullularia pullulans:

Three isolates resembled the type species (de Bary) Berkhout, described by Wynne and Gott (1956), both morphologically and in its ability to ferment sugars. Some slight differences in the macroscopical appearance of growth were apparent between(BY1, BY2) and 117A; the latter isolate produced somewhat larger blastospores than/



(a) Early stages



(b) Later stages

FIGURE 6 Strain 505, Metschnikowia krissii, on carrot plug prior to spore formation.

than the other two. All three isolates were able to assimilate the five sugars of Lodder and Kreger-van Rij (1952) as well as ethanol, but BY1 and BY2 assimilated nitrate. Arbutin was not split. No chlamydospores, arthrospores or cladosporium were observed in the young cultures maintained for three days at 25°C on malt wort agar and in malt wort. Black pigment was produced aerobically, although somewhat slowly.

X) Unidentified strains:

1) Isolate 132: It was found very difficult to assign this strain to any species described by Lodder and Kreger-van Rij (1952) and no new species resembling the isolate has been described in the literature. The isolate most nearly resembled the genus Pichia in view of its poor fermentative ability, its cell shape, its ability to produce pseudomycelia and its production of round, smooth-walled spores, held in oval or elongated-oval asci on Goradkowa's agar. However, pellicle formation did not occur in malt wort and this is considered by most authorities to be an important taxonomical characteristic of the genus. It must be noted, however, that Phaff (1956) recommended that strains other than those able to produce pellicles in malt wort should be included in Pichia.

Pichia farinosa is the species most closely resembled by this strain but differences are obvious in the absence of a pellicle in malt wort cultures of the latter, its ability to assimilate not only glucose/

glucose but galactose, maltose and sucrose, and its inability to split arbutin, even weakly.

2) Isolate 179: This isolate was also difficult to classify. Its morphology agreed most closely with that of Debaryomyces or Hansenula species, but certain features of its physiology agreed with neither i.e. no species of Debaryomyces can assimilate nitrate and all species of Hansenula ferment sugars. However its production of round, thick-walled, somewhat warty spores with central oil-droplets contained (1 to 4) in round asci on Goradkowa's agar seemed typical of Debaryomyces and it was decided to place the isolate provisionally as an unamed new species of Debaryomyces.

Conclusion

Two hundred and thirteen of the two hundred and thirty-five isolates studied were from newly caught fish and have been assigned to the genera and species indicated in Table 19.

During the course of the above taxonomical study other yeast isolates acquired from a variety of marine sources apart from fish were also studied and these have been classified as indicated in Table 20.

The diagnostic characteristics of the majority of the isolates agreed closely with those of the type species but as is to be expected when dealing with a large number of isolates differences in properties/

TABLE 19

Identification of yeasts isolated from fish:-

Species	No. of Strains	% of total Isolates
Debaryomyces kloeckeri	96	45
Torulopsis inconspicua(var)	24	11
Candida parapsilosis	23	11
Rhodotorula glutinis var. rubescens	14	6.5
Debaryomyces subglobosus	9	4
Candida lipolytica(var)	8	3.5
Rhodotorula mucilaginosa	7	3
Candida species	6	3
Torulopsis famata	4	2
Torulopsis candida	4	2
Torulopsis inconspicua	4	2
Rhodotorula rubra	3	1.5
Pichia membranaefaciens	2	1
Pullularia pullulans	2	1
unidentified	2	1
Candida zeylanoides	1	0.5
Trichosporon pullulans	1	0.5
Cryptococcus diffluens	1	0.5
Rhodotorula minuta	1	0.5
Torulopsis pseudaria (Nisolt, 1953)	1	0.5

TABLE 20

Identification of yeasts isolated from marine sources other than fish:-

Strain No.	Source	Classification
504	Sea-water, Clyde Estuary	Debaryomyces kloeckeri
68	Trawl-net, " "	" "
Light I, II	Seaweed, Aberdeen	Candida parapsilosis
40red	Winkle in rock pool, Aberdeen	Rhodotorula glutinis var. rubescens
Her3, Her7	Spoiling herring(smoked)	Candida lipolytica(var)
12pink	Seaweed, Aberdeen	Rhodotorula mucilagi-:nosa
67	Fishing boat deck, Clyde Estuary	Torulopsis famata
T40	Winkle in rock pool, Aberdeen	Torulopsis candida
507	Sea-water, Clyde Estuary	Cryptococcus diffluens
510	" " "	" albidus
511	" " "	" laurentii
43/6	" , North Sea	Torulopsis pseudaria (Zsolt, 1958)
501, 505, 506, 508, 509	" , Clyde Estuary	Metschnikowia krissii (vanUden & Castelo-Branco, 1961)
117A	" , North Sea	Pullularia pullulans
502, 503	" , Clyde Estuary	Trichosporon cutaneum var. multisporum

properties occurred which did not always make identification easy. These difficulties together with evidence from the routine tests of Lodder and Kreger-van Rij(1952) which could indicate possible strain variation within the different species have therefore been discussed.

SUPPLEMENTARY TESTS

Introduction

As mentioned earlier (see page 72) tests for the investigation of characteristics of the yeasts not determined by the methods of Lodder and Kreger-van Rij (1952) have also been undertaken. The results of these tests for the strains in each species are indicated in the Appendix. These results together with the results of earlier taxonomical investigations have been considered with a view to the division of the isolates into various groups in order to indicate strain variation. The methods used for these tests and the results obtained from them will now be discussed.

Temperature Relations

In determining growth responses of the yeast strains at various temperatures it was hoped not only to observe strain variations but any possible phenomenon relating to the marine habitats of the strains. Phaff et al.(1952) found considerable differences in temperature tole-:rance amongst different yeast isolates from shrimp, some being capable of growth at 2.5°C while others could grow at 42°. One of their isolates was found to have a narrow tolerance range - growing poorly at 10° and not at 32°. Roth et al.(1962) found that yeasts isolated from water samples at temperatures of 4° to 10°C could grow success-:fully at these temperatures and also that duplicate samples of these waters incubated at 6°C and 24°C exhibited identical total counts and species/

species composition although growth at the lower temperature was much retarded.

For this investigation "MYGP" broth cultures held in test-tubes were incubated in a stationary position at 4°C, 14°C, 25°C, 30°C, 37°C and 42°C, and the number of days necessary for the production of moderate, good and very good growth noted. Cultures autoclaved at various stages of growth were used as arbitrary standards for comparison. As can be seen in Table 21, most of the isolated strains were capable of growing over a wide range of temperature. Only 6.5% were found incapable of growing at 4°C. More variation is obvious, however, amongst the strains with regard to the maximum temperature permitting growth, i.e., 1.0% were incapable of growing above 25°C, 42% did not develop above 30°C and only 5.0% of the remaining 57% capable of growing at 37°C exhibited growth at 42°C.

TABLE 21

Percentage of yeasts growing within various temperature ranges:-

Temperature Range (°C)	4-37	4-30	14-42	14-37	4-42	14-30	4-25
% Strains exhibiting growth	50.0	41.0	3.5	2.0	1.5	1.0	1.0

No characterisation of the various species comprising the collection/

collection by the ranges of temperature permitting growth was obvious but within many of these species strain variation in this respect was found which in some cases bears a correlation to variations in other properties, e.g. T.inconspicua, T. famata.

The majority of the strains exhibited maximum growth, i.e., with respect to quantity and rate, in the range 25°C to 30°C. Strains 419H, 420H, 421m and 424H, labelled Candida species, were found, however, to grow similarly at both 14°C and 25°C and their optima may, therefore, lie between these two temperatures. A lower optimum of 14°C was exhibited by all four strains of Cryptococcus, i.e., 407, 507, 510 and 511 and hence these strains can be considered truly psychrophylic. Strain 511, Cryptococcus laurentii, was also found unable to grow above 25°C and exhibited rather poor growth at this temperature; for this reason most of the diagnostic tests for this strain were carried out at room temperature (17°C - 20°C). The occurrence of this psychrophylic strain in the only sea-water sample incubated at 4°C indicates the possible existence of an extensive yeast flora in marine waters adapted to or dependent upon lower temperatures for growth and survival and an investigation of such a flora should prove an interesting topic for future research on marine yeasts.

Assimilation of an Additional Range of Carbon Compounds

The use of an extensive number of carbon compounds for assimilation/

assimilation tests was first suggested by Wickerham (1951) and this practice has gained wide acceptance in recent years as indicated by the many published descriptions of yeasts containing the results of tests using such a range of compounds.

Using the "replica plating technique" already described for the routine assimilation tests of Lodder and Kreger-van Rij (1952) - see page 76 - the assimilation patterns of the different strains using xylose, raffinose, L-arabinose, D-mannitol, mannose, salicin, sorbitol, glycerol, inulin, laminarin and fucose were investigated. The two last-named compounds were used in the tests in view of their occurrence in seaweeds. Great differences were obvious between the various species in their ability to assimilate these compounds.

Within many of these species, e.g., D.kloeckeri, D.subglobosus, T.candida, T.inconspicua (var), C.lipolytica (var), all the strains exhibited identical assimilation patterns but within other species, e.g., C.parapsilosis, T.inconspicua, Rh.mucilaginosa, Rh.glutinis var. rubescens, widely varying results in this respect were obtained and these differences have been used as criteria for placing the strains of these species in groups as indicated in the Appendix.

It seems, therefore, that the use of a large range of carbon compounds in assimilation tests could be useful in future studies of marine yeasts both as an aid to species identification and to reveal

any/

any strain variation existing within these species.

Liquefaction of Gelatin

In general yeasts have been found to possess only poor ability to break down extracellularly proteins and polypeptides (Harris, 1958: Wickerham, 1951) and for this reason Lodder and Kreger-van Rij (1952) include in their monograph the ability to liquefy gelatin only as a confirmatory test for some species, e.g. Candida lipolytica.

However, it was decided to test the ability of all the yeast strains to liquefy gelatin in view of the reports of Dyer (1947) that most of the yeasts isolated by her from Atlantic cod were able to attack fish protein, and of Kriss (1959) who found that strains of most of the species comprising his marine isolates were able to liquefy gelatin.

As can be seen in the Appendix the ability to liquefy gelatin was not a common property of these marine isolates. Apart from the strains of C.lipopytica (var) resembling C.lipolytica for which this ability is a characteristic listed by Lodder and Kreger-van Rij (1952) only strains 419H, 420H, 421m, 424H and 427s (Candida spp.) and 502 (Trichosporon cutaneum var. multisporum) were found capable of lique-:fying gelatin. . It is interesting to note that all these strains producing proteolysis were also liplytic (see below).

Lipolysis

As/

As in the case of gelatin liquefaction, Lodder and Kreger-van Rij (1952) apply a test to detect fat-splitting in only a few cases for confirmation, viz., in Candida lipolytica and Trichosporon pullulans. The method recommended for the test by these workers has, however, not proved very satisfactory (Morris, personal communication) and therefore that developed by Willis (1960) to detect lipolysis by Clostridia was adopted instead. This consists of streaking the organisms onto the surface of a medium composed of one part cow-cream and nine parts "MYGP" agar (autoclaved separately and mixed at about 50°C prior to pouring) held in petri-dishes. After four days' incubation at 25°C the plates are flooded with a saturated solution of $CuSO_4$ and allowed to stand twenty minutes before pouring off the excess. Lipolysis is indicated by the formation of a dark greenish-blue colour both on the streak and round about it which is due to the formation of insoluble copper soaps by copper sulphate with any released fatty acids.

All the strains in the collection were tested for lipolysis in this manner. Strong colour formation was observed with the strains of C.lipolytica(var) and 1113, Trichosporon pullulans. Somewhat weaker reactions were also observed for 502 and 503 (Tr.cutaneum var. multisporum), 43/6(T.pseudaria), 40red (Rh.glutinis var. rubescens) and 421m, 424H, 419H and 427s (Candida spp.)

Tests were also made using 5% commercial herring oil (filter-sterilised)/

sterilised) in place of the cream and with this only a slight colour reaction was obvious from the strains of C.lipolytica (var) and Candida spp., 421m, 420H, 424H, 419H and 427s. This weak reaction with herring oil may, however, be only an indication of the potential capacity inherent in these strains for spoilage, i.e., with regard to lipolysis, of fish materials which may be more apparent under a different set of conditions, e.g., lower temperatures, different pH, different media, etc. In this regard it is interesting to note that Bulder (1955) reported that a strain of Pseudomonas fluorescens exhibited lipolytic activity only at 25°C and not at 30°C, although growing well at both temperatures.

Riboflavin Production

The ability to excrete slight amounts of riboflavin into the synthetic liquid used in the assimilation tests was noted for many of the strains during this investigation. This ability was not confined to any one particular species although strains of C.parapsilosis, D.subglobosus and D.kloeckeri exhibited the property most commonly. Lodder and Kreger-van Rij (1952) specifically mention D.kloeckeri, T.famata and T.candida as having this ability and recently Siepmann and Höhnk (1962) noted particularly that many of their yeast isolates from the North Atlantic, especially those of D.subglobosus and T.candida excreted considerable amounts of riboflavin into their growth medium.

Many/

Many other reports concerning the ability of yeasts to synthesise riboflavin have been published, most of which are mentioned in a reveiw by Pridham (1952) and these deal mainly with attempts to establish optimum conditions for riboflavin production. In exactly defining these conditions some controversy exists, but it was generally agreed that a chemically defined medium containing inorganic salts, glucose, amino-acids and vitamins, is suitable for the purpose.

In this study it was hoped that some strain differentiation would be possible on the basis of riboflavin excretion and hence it was necessary to produce measurable amounts of riboflavin in a reproduceable manner. Initial experiments with strain 120, C.parapsilosis were made using carbon assimilation medium (Lodder and Kreger-van Rij, 1952) with 1% glucose and 1% concentrated vitamin solution (Lodder and Kreger-van Rij, 1952) minus the riboflavin. Flasks containing this medium alone or with the addition of 0.1% "A-Z" trace element solution (Meiklejohn, 1950) were inoculated with a washed suspension of cells from a three-day-old "MYGP" culture and then placed at 25°C, one set being shaken and the other left stationary. After three days' growth the amount of riboflavin excreted into the medium in each culture was estimated spectrophotometrically (Brealey and Elvidge, 1956). The highest result, i.e., approximately 2µg/ml was obtained from the shaken culture containing the added trace elements.

Fourteen/

Fourteen other yeast strains comprising nine D.kloeckeri, four C.parapsilosis and one T.candida were then tested for riboflavin production using shaking cultures with the latter medium and a parallel set was also put up containing in addition asparagine and glycine, 2g/litre, respectively. However, although bright green fluorescence was produced by all the strains under U.V. light, only strain 120 excreted measurable amounts of riboflavin and it was concluded that under these conditions no strain differentiation on the basis of riboflavin production would be practicable. However, further elucidation of the metabolic pathway of riboflavin production and its role in yeast physiology may prove helpful for future comparative studies in this field.

Vitamin Requirements

Introduction:

The determination of vitamin requirements is possibly one of the most commonly employed methods for the recognition of different strains within the same species of yeast and hence it was decided to determine this characteristic for most of the marine isolates. The methods used for this study were based on those of Schultz and Atkin (1947) and Kirsop (1955) and as some variation in nutrilite requirement under different environmental conditions has been reported (Morris, 1958) a description of the standard method employed is given below.

Methods:

The mediumwas prepared according to the methods of Schultz and Atkin/

Atkin (1947) except that the concentrations of the vitamin solutions were changed to those indicated below, these values having been found to give improved results (Morris, personal communication).

Vitamin Solution

Inositol	- 0.625	g/litre
Calcium pantothenate	- 0.25	g/litre
Biotin	- 0.1	mg/litre
Thiamine hydrochloride	- 12.5	mg/litre
Pyridoxine hydrochloride	- 12.5	mg/litre
Nicotinic acid	- 12.5	mg/litre

Para-amino-benzoic acid was also used in the test and a solution containing 1.25 mg/litre was made up and sterilised similarly to the nicotinic acid solution.

Inocula were prepared from three-day-old cultures (grown on "MYGP" agar at 25°C) by washing off the growth into sterile bottles and making from these suspensions containing 1 x 10^6 cells per.ml. The nine different media required for a test were held in bent test-tubes and these were each inoculated with 0.5 ml of the standard cell suspension of the organism under investigation. The cultures were then fitted in a sloping position on a tray which was placed on a shaker (60 cycles/min.) at 25°C.

After twenty-four, forty-eight and seventy-two hours, estimations of the growth in the cultures were made by placing the tubes in an Eel absorptiometer with a neutral density filter using water in a similar tube as a blank. Readings were taken from the transmittance cale.

Complete/

Complete growth was reported if 95% absorption occurred. If, after seventy-two hours, however, less than 50% absorption was indicated the particular vitamin missing from this culture was reported as "essential" and if the absorption lay between 50% and 95% this was reported as "partial requirement".

Results:

The results for the individual strains can be seen in the Appendix and these have been summarised for the different species in Table 22. As can be seen in this table, considerable variation exists amongst the strains of many species and it has been possible to use the different requirements as criteria for the grouping of strains within the species (see Appendix). Strains of D.kloeckeri, D.subglobosus, C.lipolytica(var) and M.krissii, however, proved consistent in their requirements and strain differentiation for these species on this basis was impossible.

Candida spp., T.pseudaria, Rh.glutinis var. rubescens, Rh.rubra, Rh.mucilaginosa, P.membranaefaciens and Tr.pullulans all had strains which could grow without any vitamin supplements. It is difficult to relate this independence from vitamins of these strains to their marine occurrence in view of the widespread marine occurrence of the vitamin-dependent strains.

Recently Ahearn and Roth (1962) have published the results of tests concerning the nutrilite requirements of approximately three hundred/

TABLE 22

Vitamin requirements

Yeast species	Number tested	Vitamin requirements *
D.kloeckeri	97	B
D.subglobosus	9	B
C.parapsilosis	25	18 - B; 1 - B,Th,Pyr; 1 - B(p),Pan(p),Pyr(p) 1 - B(p); 1 - B,Th,PAB; 1 - B,In,Th(p),Pyr; 1 - Pyr(p); 1 - B,Th(p)
C.zelanoides	1	B
C.lipolytica(var)	10	Th
Candida spp.	6	5 - none; 1 - B
T.inconspicua	4	2 - Nic,Pyr,Th,B; 1 - B,Th,Pyr(p),PAB(p); 1 - B,Pyr,Th(p)
T.inconspicua (var)	24	11 - B,Pyr; 8 - B,Th(p),Pyr; 4 - B,Pyr(p)
T.candida	4	2 - B; 1 - Th(p); 2 - B,Th
T.pseudaria	2	none
T.famata	5	4 - B; 1 - B,Th(p)
Rh.glutinis var. rubescens	15	6 - none; 3 - Th(p); 6 - Th
Rh.mucilaginosa	8	3 - none; 3 - Th(p); 2 - Th
Rh.rubra	3	1 - none; 2 - Th
Rh.minuta	1	1 - Th(p)
M.krissii	5	B
P.membranaefac--iens	2	none
Cryptococcus spp.	4	2 - none; 1 - Th; 1 - B(p)
Tr.pullulans	1	none
Tr.cutaneum var. multisporum	2	Th(p)

(p)- partial requirement B - Biotin Th - Thiamine Pyr- Pyridoxine

Nic - Nicotinic acid In - Inositol Pan - Calcium pantothenate

PAB - p-Aminobenzoic acid

* Number preceeding requirement indicates the number of strains

hundred marine isolates, the collection of which has been described by Fell et al (1960) and Roth et al (1962). As in this test biotin was found to be the vitamin most commonly required amongst representa-:tives of the genera Candida, Debaryomyces and Torulopsis and thiamine amongst those of Rhodotorula. It is also interesting to note that these workers when comparing yeasts of the same genera from marine and terrestrial sources detected no obvious differences in their vitamin requirements. In view of the many different marine locations from which the latter yeasts were isolated and the different types of response obtained from them the latter workers concluded that yeast distribution in true marine habitats is not influenced significantly by specific vitamin demands and this is also the finding in the present study with regard to the type of fish and fishing ground from which the strains were isolated.

Conclusion

Determination of vitamin requirements and of ability to assimilate a large range of carbon compounds as well as investigation of tempera-:ture relations proved the most useful of the supplementary tests in providing criteria for the division of the isolates into groups in order to indicate strain variation. Determination of the ability to split fat and of ability to liquefy gelatin were not so useful in this regard. No practicable method could be devised for comparative estimations of riboflavin production.

The wide ranges of temperature permitting growth of these yeasts have/

have been indicated and the presence of several psychrophylic strains was revealed. Lipolytic activity was found not only in strains of C.lipolytica (var) and Trichosporon spp., but in those classified as Torulopsis pseudaria, Rh.glutinis var.rubescens and Candida spp.. The presence of this ability in strains of Torulopsis and Rhodotorula has not, as far as is known, been previously reported in the literature. It was also noted that all the strains producing proteolysis, i.e., liquefying gelatin, were able to cause lipolysis. Neither lipolysis nor proteolysis was, however, a general property of these marine isolates.

INFRA-RED SPECTROPHOTOMETRY

Introduction

Infra-red spectrophotometry which is prominent among recently developed techniques in chemistry and biology makes use of the concept that the infra-red spectrum of an organic compound is a unique property and also that a small change in the chemical constitution of that compound may produce a relatively large change in its spectrum. In more complex molecules, however, the absorption bands are somewhat diffuse but, nevertheless, molecules having similar composition have similar spectra. Hence, it has been found possible using this technique to make comparative investigations of the overall molecular composition of unicellular micro-organisms.

Stevenson and Balduan (1952) were first to apply this technique in the identification of bacteria and they found that the characteris-:tic spectra obtained not only enabled the organisms to be placed in genera and species but allowed differentiation of closely related strains. Further studies of various groups of bacteria by Norris (1953), Levine et al.(1953), Thomas and Greenstreet (1954), Riddle et al (1956), Greenstreet and Norris (1957), Norris and Greenstreet (1958), Kenner et al. (1958), Haynes et al. (1958), Goulden and Sharpe (1958) and Scopes (1962) confirmed the findings of Stevenson and Balduan (1952), although some genera were found to be easier to differentiate than others.

Other/

Other groups of organisms have also been studied by these methods, i.e., viruses by Benedict et al. (1954) and pathogenic fungi by Cawley et al. (1954), but in either case the spectra obtained afforded little strain or species differentiation. Simon and Hedrick (1955), making similar studies of the two yeast species Hansenula anomala and Saccharomyces cerevisiae were unable to obtain dissimilar spectra even when the yeasts were compared in parallel under widely varying experimental conditions. They found further, that the cellulose obtained from the yeast cells had a similar spectrum to that of the whole cells and, hence, in these species at least, cellulose must contribute substantially to the whole cell infra-red spectrum. Although relatively little is yet known of the structural polysaccharides of yeasts this finding is surprising in view of the reports of considerable variations in the composition of the cell wall fraction of different yeast species - Usden and Burrell (1952), Kreger (1954), Miller and Phaff (1958) and Klaushofer (1961). However, because Simon and Hedrick (1955) studied only two yeast species, neither of which has occurred in the marine yeast collection, it was considered profitable to explore further the possibility of using infra-red spectrophotometry in order to establish some differentiation amongst the marine isolates, especially at strain level.

It has been stressed by nearly all the above workers when employing infra-red spectrophotometry for microbiological analysis that/

that reproduceable results can be achieved only under rigidly controlled experimental conditions. In this context it was found that the spectra of micro-organisms could be affected by such factors as variations in culture media, type of culture medium and incubation time and temperature. The methods of sample preparation and operation of instrument are also important experimental details which must be controlled strictly.

The standard procedures adopted in this study, based on those of the above-mentioned workers, attempt to eliminate all the recognisable variables.

Infra-red spectra of intact cells

"MYGP" broth cultures of yeast strain 43, Debaryomyces kloeckeri, were grown on two successive occasions for forty-eight hours ($\pm \frac{1}{2}$ hour) at 25°C. A portion (0.5 ml) of the second culture was then spread evenly, using an L-shaped rod, over the surface of "MYGP" agar, i.e., 20 ml, held in 10-cm petri-dishes, and after leaving at room temperature for a quarter of an hour the inoculated plates were incubated at 25°C.

After forty-eight hours' growth ($\pm \frac{1}{2}$ hour) the cells were harvested from the plate, care being taken to avoid any "carry-over" of media. Physiological saline (1.5 ml) at approximately 4°C was added to the plates which were inclined at 10° on a rack and the growth scraped off gently with an L-shaped glass rod. The resulting suspension/

suspension was then transferred to a sterile, screw-cap bottle (¼ oz.) using a bent pipette. A few drops of this suspension were trans-:ferred to polished silver chloride plates (1"xl"xlmm), spread evenly over the surface and dried slowly to a film on a hot plate at 50°C. On the recommendation of Greenstreet and Norris (1957) attempts were made to keep the time intervals constant between removing the cell suspension from the culture medium and adding it to the silver chloride plate.

The silver chloride plate was slightly tilted while it dried so that the thickness of the film varied along its length. The infra-red spectrum was then run from wavelength 2.5μ to 15μ (i.e., wave no. 4,000 cm^{-1} to 700 cm^{-1}), using a Perkin-Elmer 137 Infracord Spectrophotometer. Films giving 40/80% transmission at wavelength 2.5μ were found to produce satisfactory spectra. Before each recording the chart scale was correctly aligned using a polystyrene film of known infra-red spectrum.

From different preparations of strain 43, both on the same and different occasions, identical spectra were obtained. These spectra were compared on the basis of the position and shape of the absorp-:tion bands rather than their intensity which varies with each sample, depending on the thickness of the film - a factor which is difficult to control. As can be seen from a representative tracing of the spectrum of strain 43 (Figure 7) the main absorption bands occur at 6.05μ,/

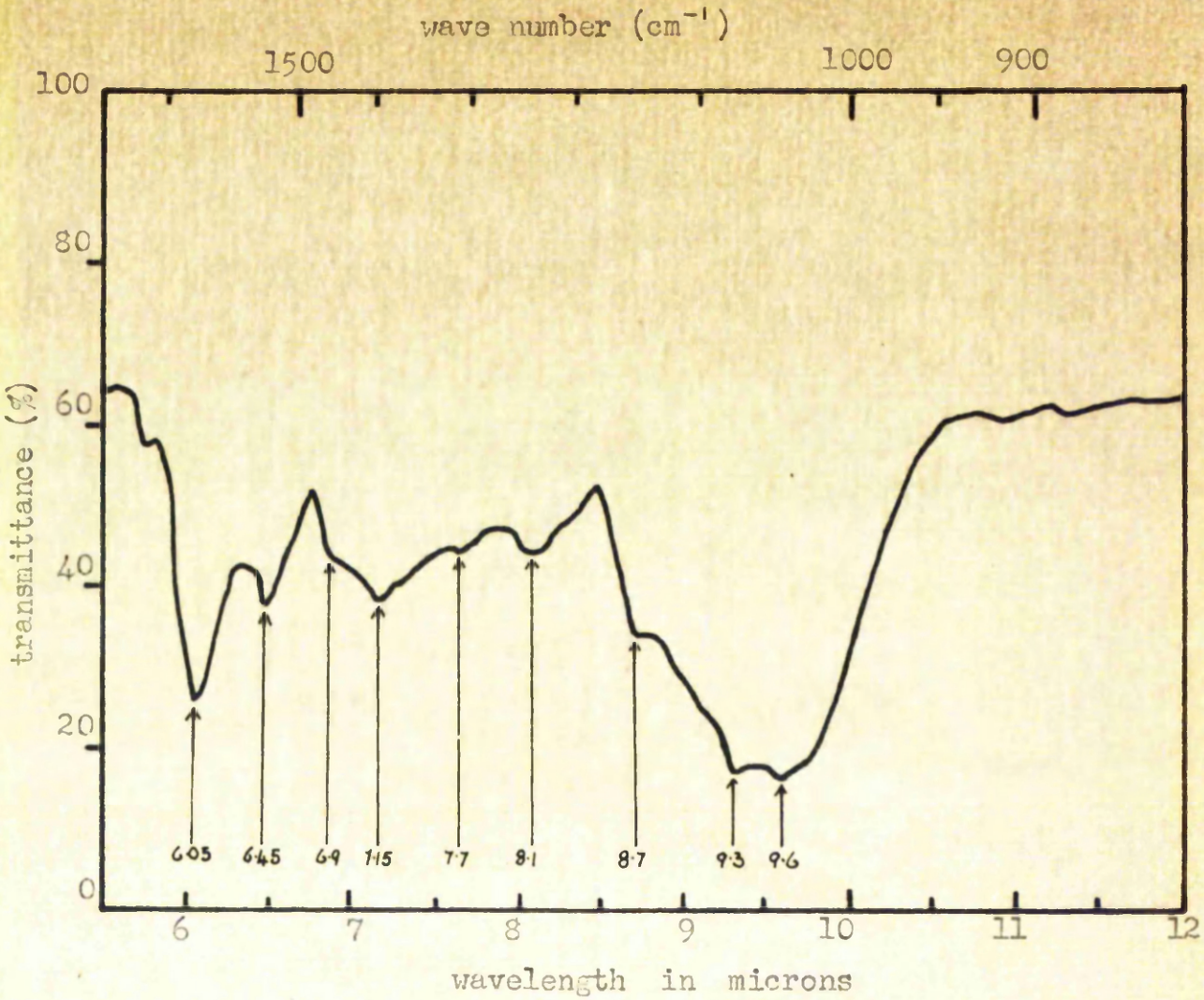


FIGURE 7 Infra-red spectrum of whole cell preparation of strain 43, Debaryomyces kloeckeri.

6.05μ, 6.45μ, 7.15μ, 8.1μ and 9.3/9.6μ, with weaker ones at 6.9μ, 7.7μ and 8.7μ.

The spectrum of strain 43 is very similar to that obtained by Simon and Hedrick (1955) for whole cells of Hansenula anomala except for small differences in the position of two bands which they recorded at 7.0μ and 8.0μ and in the spectrum of strain 43, bands occurred at 7.15μ and 8.1μ. These may well be the same absorption bands, however, as the tracing of the spectrum published by these workers has very shallow absorption bands and their exact position seems rather indeterminate. The marked intensity of the absorption band occurring between 9.3μ and 9.6μ in strain 43 was also noted in the spectrum of Hansenula anomala by these workers who demonstrated that its occurrence was largely due to yeast cellulose.

Yeast strains 42, Torulopsis candida, and 21, Candida parapsilosis were also similarly examined by infra-red spectrophotometry both at the same time as strain 43 and on different occasions and in all cases the spectra obtained were found to be identical to that of strain 43 and hence to each other (see Figure 8). It thus appears that infra-red spectrophotometry of whole yeast cells will not allow differentiation of these yeasts at any taxonomical level.

Infra-red spectra of disrupted cells

The finding of identical infra-red spectra for different yeast species/

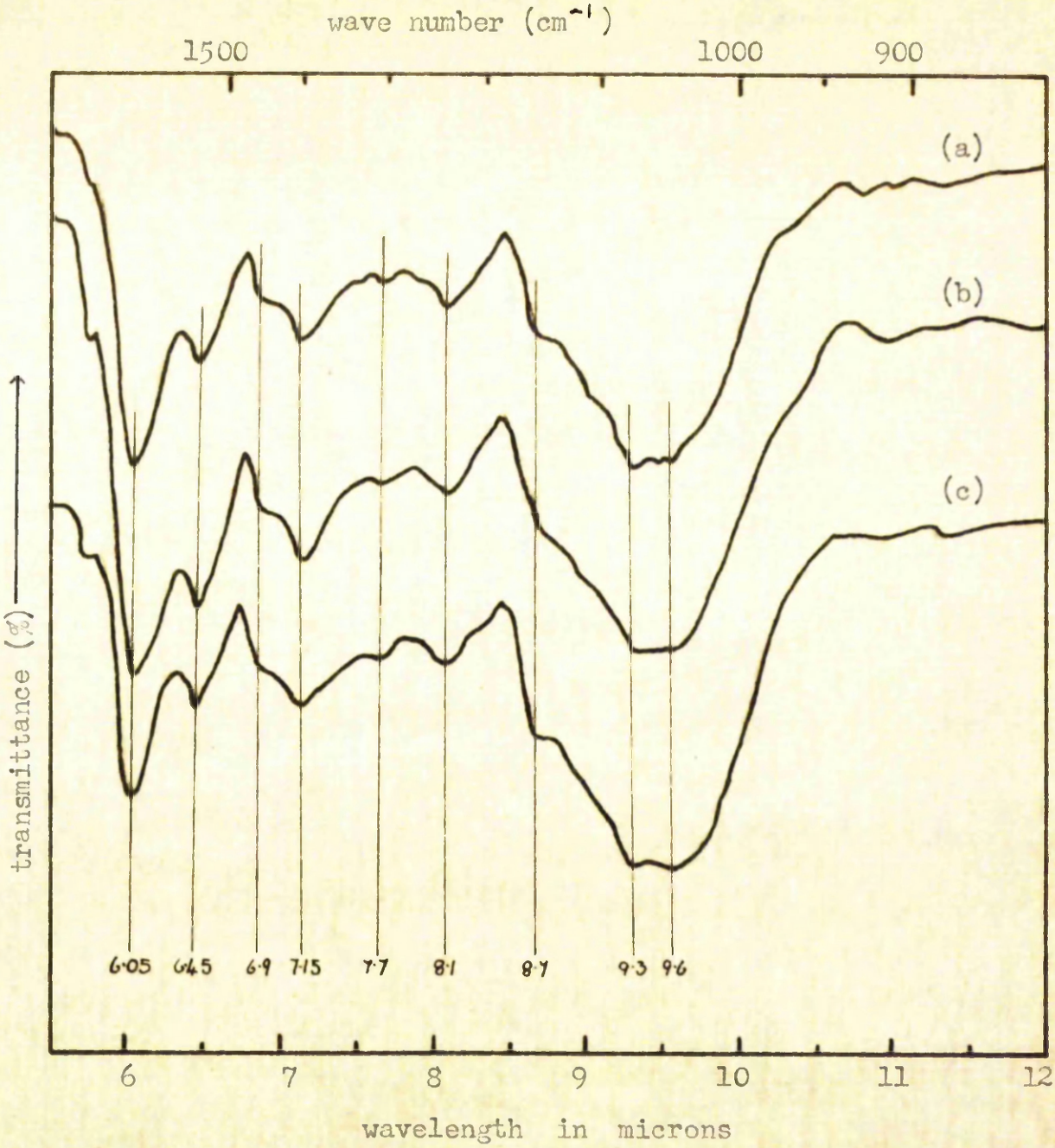


FIGURE 8 Infra-red spectra of whole cell preparations of:-

(a) strain 21, Candida parapsilosis

(b) strain 42, Torulopsis candida

(c) strain 43, Debaryomyces kloeckeri

species confirms the results of Simon and Hedrick (1955) with Hansenula anomola and Saccharomyces cerevisiae and as these workers demonstrated that yeast cellulose from both the latter species produced similar spectra which in turn were similar to the whole cell spectra it was decided that an examination of the yeast cellular material after the removal of a substantial part of the cellulose might afford characteristic spectra.

Cells were therefore harvested as previously, although in this instance "MYGP" medium was replaced by a synthetic medium described by Lodder and Rij (1952) for testing the assimilation of glucose. This change of medium was thought necessary as slight changes in batches of such an undefined medium as "MYGP" could be reflected strongly in the intracellular chemical composition and might become apparent once the masking effect of the cellulose was removed.

The cells were then disrupted by vibration with No. 13 Ballotini shot in a Mickle disintegrator and the insoluble cellular material removed by centrifuging at 2,500 r.p.m. Mechanical disruption was preferred here rather than chemical reagents which are difficult to remove and small traces of these can affect the spectra. The resulting opalescent supernatant was then freeze-dried with 100 mgm specially purified KCl and the fine white powder obtained pressed into discs from which spectra were recorded as previously.

Yeast strains 42, 43 and 21 were examined by this method and again identical spectra were obtained (see Fig. 9). These spectra differ/

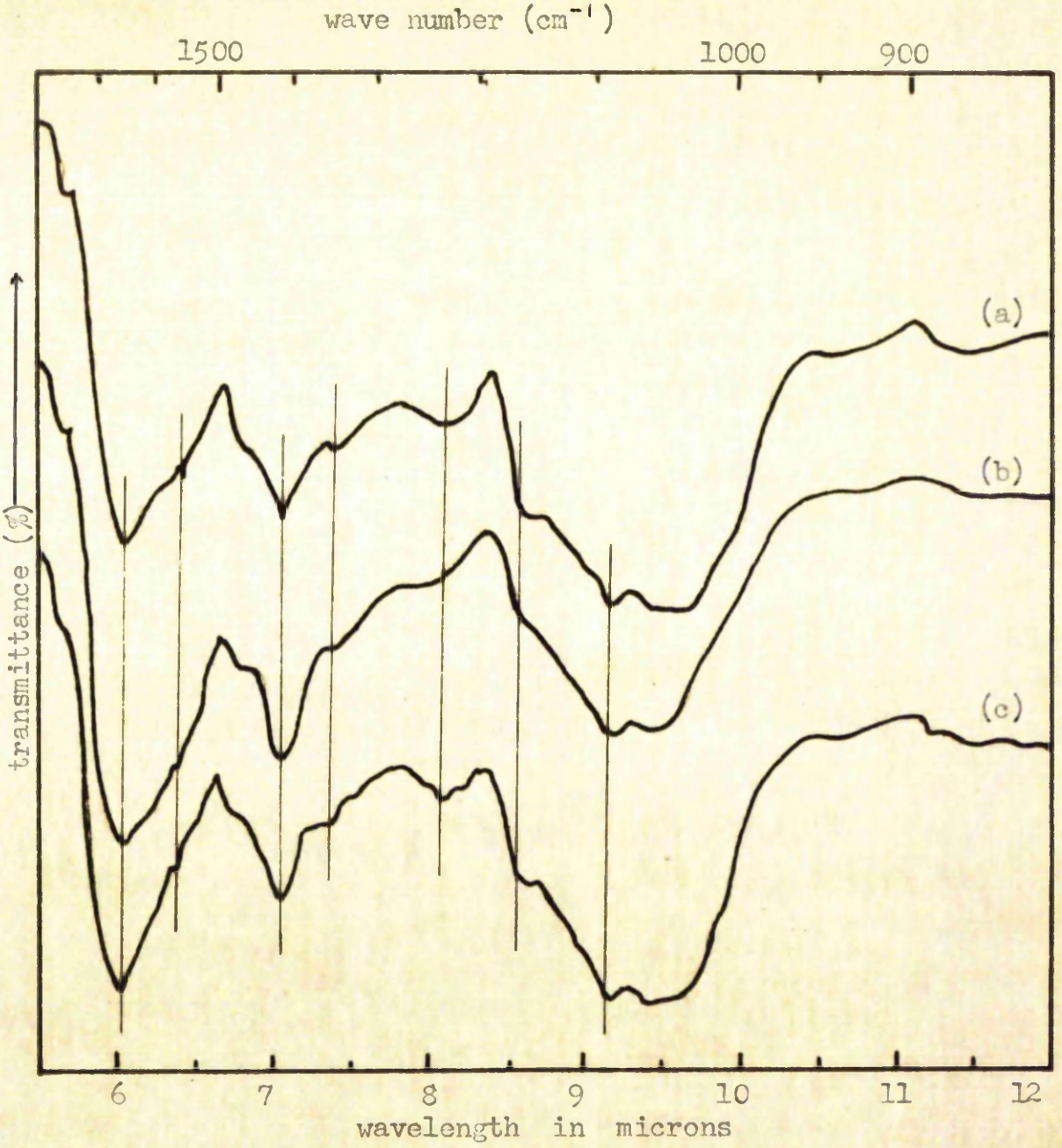


FIGURE 9 Infra-red spectra of soluble material from disrupted cells of:-

(a) strain 43, Debaryomyces kloeckeri

(b) strain 21, Candida parapsilosis

(c) strain 42, Torulopsis candida

differ from those of the whole cells only in having a very slightly perceptible band of absorption at 6.45μ, which is considered to be a protein band (Levine et al., 1953); but no difference in the intensity of the bands 9.3μ to 9.6μ, considered by Simon and Hedrick (1955) to be the cellulose bands, was found.

Further investigations of strains 43 and 21 were carried out employing a much higher speed of centrifugation - 35,000 "g". This reduced the opalescence of the supernatant and it was hoped that most of the yeast cellulose would thus be removed. Both supernatant liquid and the deposit as well as the whole cells of strains 43 and 21 were freeze-dried simultaneously with KCl and the resulting powder used for discs to determine the spectra, as before.

As can be seen in Figure 10, although the spectra of the whole cells, deposit and soluble material are somewhat different, the spectra in each case for the two strains 43 and 21 were very similar. The production of similar spectra by the soluble cellular material, as well as by the insoluble in the different strains seems to suggest that some component, other than cellulose, present in relatively large amounts in each strain, produces a characteristic spectrum which masks any effects of other cellular constituents. In this connection it is interesting to note that the spectrum obtained from bacterial glycogen by Levine et al. (1953) somewhat resembles the spectrum obtained here from the soluble cellular material, especially in the intensity of the absorption band between 9μ and 10μ. Thus, it can be conjectured/

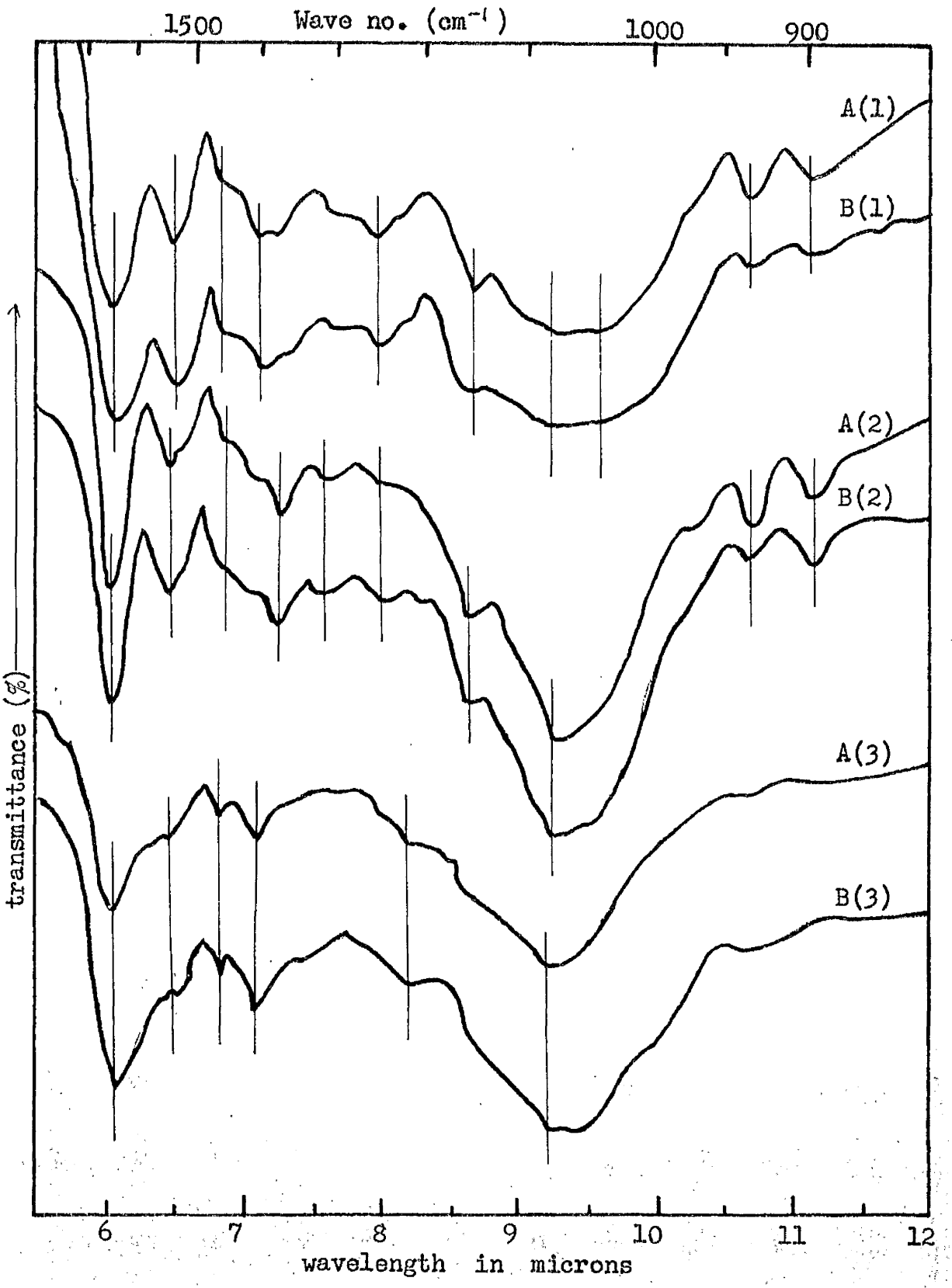


FIGURE 10 Infra-red spectra of:- A) strain 21, C.parapsilosis
B) strain 43, D.kloeckeri
(1) whole cells (2) deposit (3) supernatant

conjectured that both cellulose and probably glycogen, which occur in all the yeast strains, produce specific spectra which mask any effects of the differences in the other cellular components which could otherwise produce characteristic spectra. It thus appears that differentiation of these yeasts, at any taxonomical level, by infra-red spectroscopy will not be possible until the components masking the effects of the characteristic overall intracellular chemical composition are removed from the cells. Removal of these components would require the use of chemical reagents, a procedure which is not desirable in view of the possible difficulty of removing traces of such and also the introduction of further complications, e.g.denatura-:tion of protein, etc. Hence variables could be introduced which would decrease the chances of obtaining reproduceable results. No further attempts to differentiate the marine isolates were, therefore, made, except for those of the genus Rhodotorula, as reported below.

Infra-red spectra of Rhodotorula species

In view of the difficulties of obtaining criteria for the alloca-:tion of species in the genus Rhodotorula (Skinner and Huxley, 1956) a final attempt to test the possibility of infra-red spectrophotometry for this purpose was made, using the marine isolates, 330p Rh.mucilaginosa, and 302p, Rh.glutinis var.rubescens.

Kreger (1954) found that Rh.glutinis lacked both mannan and glucan in the cell walls, components which may cause at least part of the masking effects described above and it was hence hoped that any differences/

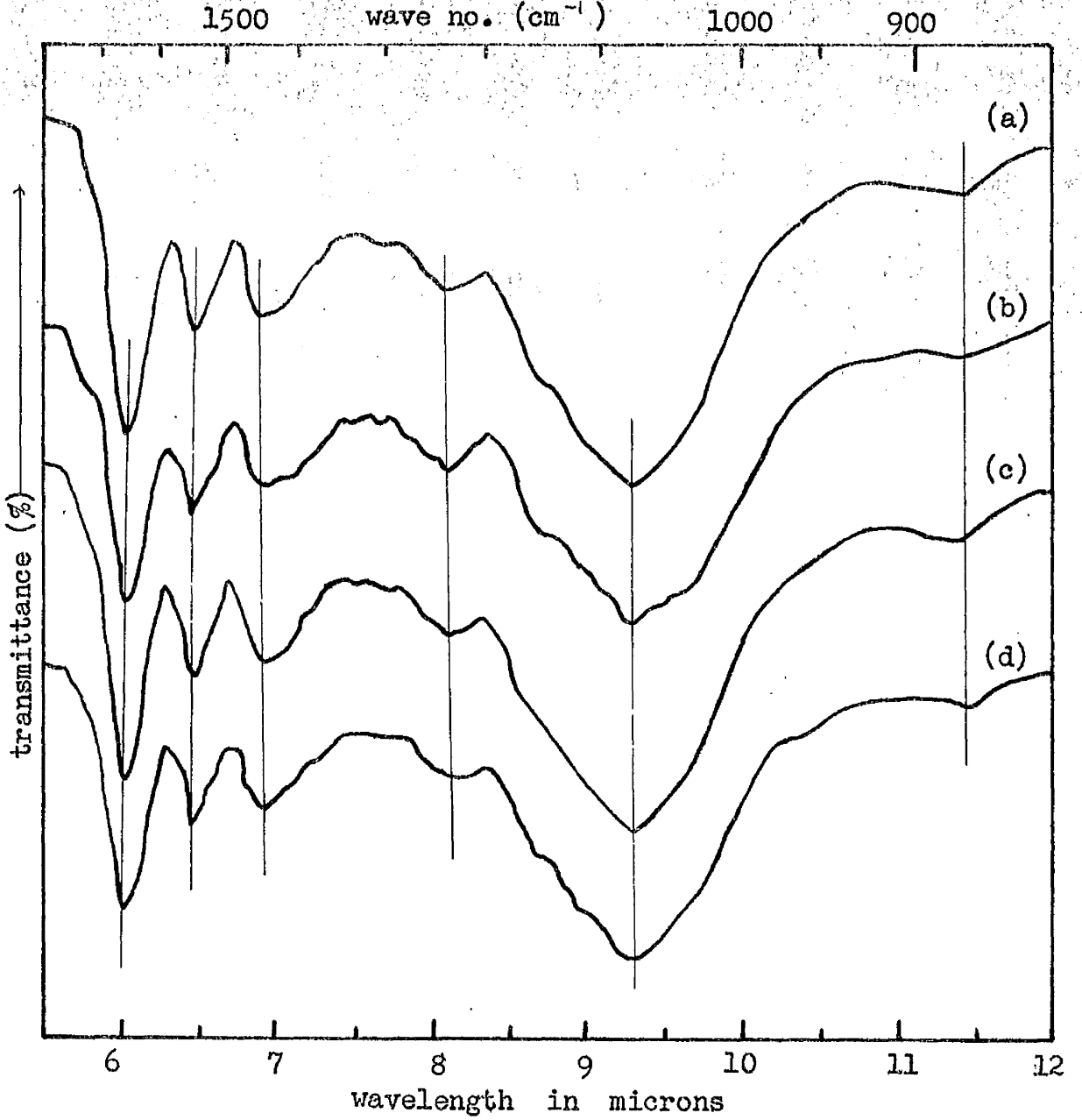


FIGURE 11 Infra-red spectra of whole cell preparations of:-

(a) 302p, Rh.glutinis var.rubescens, grown with mannose
(b) " " " " " , " " glucose
(c) 330p, Rh.mucilaginosa, grown with mannose
(d) " " " , " " glucose

differences in intracellular chemical composition between these species would be obvious in their infra-red spectra. The organisms were grown on the synthetic media and also on similar media containing mannose instead of glucose. A different carbon source, i.e., mannose, was included in the growth medium in order to see if more distinctive spectra could be produced as a result of some possible modification in the intracellular polysaccharides.

Whole cell spectra were then obtained as before and, as can be seen in Figure 11, the spectra of cells from both strains, 302p and 330p, grown with either glucose or mannose, were exactly similar. It seems, therefore, that infra-red spectrophotometry will not serve to differentiate or characterise these species of Rhodotorula and, like the other yeast strains studied, some cellular fractionation may be necessary to obtain distinctive spectra.

Conclusion

The use of infra-red spectrophotometry in the study of the yeast isolates has been investigated and the results indicate that the spectra of whole cells, soluble and insoluble cellular material, are of little value for species or strain differentiation. It is conjectured that identical cellular components producing specific spectra occur in the different species and mask the effects of differences in other cellular components.

DETERMINATION OF FREE AMINO ACIDS

Introduction

Taylor (1947) first reported the occurrence in yeasts of a free amino-acid pool containing arginine, glutamic acid, histidine, lysine and tyrosine. Other workers - Lindan and Work (1951), Nagai (1953), Halvorson and Spieglman (1953), Halvorson et al. (1955), Spieglman et al. (1955), Swenson and Dott (1961) - in further examinations of this pool indicated that at least sixteen amino acids may be present comprising from five to twelve percent., of the total cell nitrogen.

The accumulation of amino acids and peptides within the microbial cell, whether due to anabolic or catabolic processes, must be as much a reflection of the metabolism of the organisms as are sugar fermentation, assimilation of carbon-and nitrogen-containing compounds, splitting of arbutin, etc. Hence various workers - Mattick et al. (1956), Cheeseman (1959), Berridge et al. (1957) Mandelstam (1958) - have found that comparative studies of the free amino acid pools of different species and strains of bacteria may be characteristic and hence useful aids in taxonomy. For the Fungi such studies have also proved useful, e.g., Hine (1960) separated four species of Pythium on this basis. Little comparative study of the free amino acid pools of different yeast strains has been reported, however, and it was decided in view of the findings reported for bacteria and for Pythium that useful information for strain differentiation might be obtained from such an investigation.

Methods

Preparation of Cellular Material:

In view of the differences found in the free amino acid pools of yeasts grown in different media (Nagai, 1953; Halvorson et al., 1955) it seemed desirable to employ in these investigations a growth medium whose composition could be strictly controlled. A synthetic medium - medium "C" - was devised, consisting of ninety-five parts of a salts and sugar solution - solution "A" - and five parts of a vitamin solution - solution "B". Solutions "A" and "B" were prepared separate-:ly, sterilised by tyndallisation and stored at 4°C until required to prepare medium "C".

Solution "A"			Solution "B"		
$(NH_4)_2SO_4$	-	5.0 g	Biotin	-	0.04 mg
KH_2PO_4	-	1.0 g	Pyridoxine	-	5.0 g
$MgSO_4.7H_2O$	-	0.5 g	Inositol	-	0.25 g
$CaCl_2.2H_2O$	-	0.1 g	Ca pantothenate	-	0.1 g
NaCl	-	0.1 g	Nicotinic acid	-	5.0 mg
Glucose(analar)	-	10.0g	p-Amino-benzoic acid	-	0.5 mg
Distilled water to		950 ml	Distilled water to		400 ml

The yeast cells for investigation were harvested from forty-eight hour cultures grown in medium "C" held in 50 ml portions in conical flasks on a shaker at 25°C. Hence they could be regarded as being in the stationary phase of growth. The inoculum for these cultures was obtained from the second of two consecutive forty-eight hour cultures of/

of the yeast in medium"C" held in 10 ml. portions in bent test-tubes and shaken at 25°C. Prior to inoculation the medium"C" held in the conical flasks and test-tubes was sterilised in flowing steam for half an hour.

Approximately 15 x 10^9 cells were harvested, washed three times with glass-distilled water using centrifugation and then disrupted with Ballotini shot (No.13) in a Mickle Disintegrator. The resulting suspension was poured off, centrifuged for an hour at approximately 2,000 r.p.m. and the supernatant mixed with four times its volume of acetone(analar) to precipitate any remaining protein. This was removed by filtration through Whatman's No.42(acid-washed) filter paper and the filtrate so obtained evaporated to dryness under a vacuum.

The dried material was reconstituted for examination by adding approximately 10 ml. glass-distilled water. This solution was then allowed to run through an acidified resin column containing"DOWEX" 50W". The column and its contents were then thoroughly washed with glass-:distilled water to remove salts after which the adsorbed materials were eluted with redistilled $\bar{N}$ ammonium hydroxide. This solution was evaporated to dryness under vacuum after which as much of this dried material as possible was taken up in 0.5 ml. glass-distilled water and placed on a clean plastic plate held in an evacuated desiccator.

After/

After approximately twelve hours some of the resulting concentra-:ted dried material was removed with a few drops of glass-distilled water using a capillary pipette and used to 'spot' the chromatographic paper.

Preparation of Chromatograms:

A two dimensional paper chromatographic system devised by Redfield (1953) proved advantageous for the purposes of this investigation in making possible the separation of a large number of amino acids on a single chromatogram within twenty-four hours. As the papers are deve-:loped with ascending solvent systems it was possible by using a frame to accommodate simultaneously a number of them in the one tank. By the inclusion of a paper with a standard mixture of amino acids together with those containing the cellular material direct comparison of the spots obtained was possible in each batch thus lessening the need to control rigidly such factors as temperature, time etc. which can affect rf values.

The chromatographic paper best suited for this technique is Whatman's No.20 (Patterson,personal communication) and was used in this instance in squares (19.6 cm x 19.6 cm) with holes cut in each corner for their accommodation on the frame. The spot containing the cellular material or standard solution was placed in one corner of the paper $1\frac{1}{2}$" from each margin. A mixture of methanol/water/pyridine(80/20/4) was used for the first dimension. Satisfactory separation was acheived after exposing the papers from four to four and a half hours. After drying/

drying at room temperature for approximately fifteen minutes the second dimension was run for sixteen and a half hours using a solvent system of tert-butanol/methyl-ethyl-ketone/water/diethylamine (40/40/20/4). On removal from the second solvent system the chroma-:tograms were allowed to dry at room temperature and then placed in a dressings' drum which was exposed to flowing steam to allow removal of any traces of adsorbed diethylamine which could make the papers too basic for good colour development. The papers were then dipped into 0.5% w/v ninhydrin solution (in acetone) and the colour allowed to develop in a warm room.

Results

An exact tracing of the chromatogram of the standard amino acid mixture is given in Figure 12. Aspartic acid and proline were easily detected showing up blue and yellow respectively. Iso-leucine could be distinguished from leucine by its pinker appearance although this was not always so obvious on the chromatograms of the cellular material. Ornithine was found to occupy a position between arginine and lysine but on the chromatograms of cellular material only a broad spot appeared in this area affording no distinction between lysine and ornithine; it was therefore labelled lysine/ornithine. Cystine which appeared as a single elongated spot on the standard chromatogram occurred as two spots joined faintly on the other chromatograms.

Altogether eighteen yeast strains, four of terrestrial origin and the rest marine isolates, comprising eight different species, were examined/

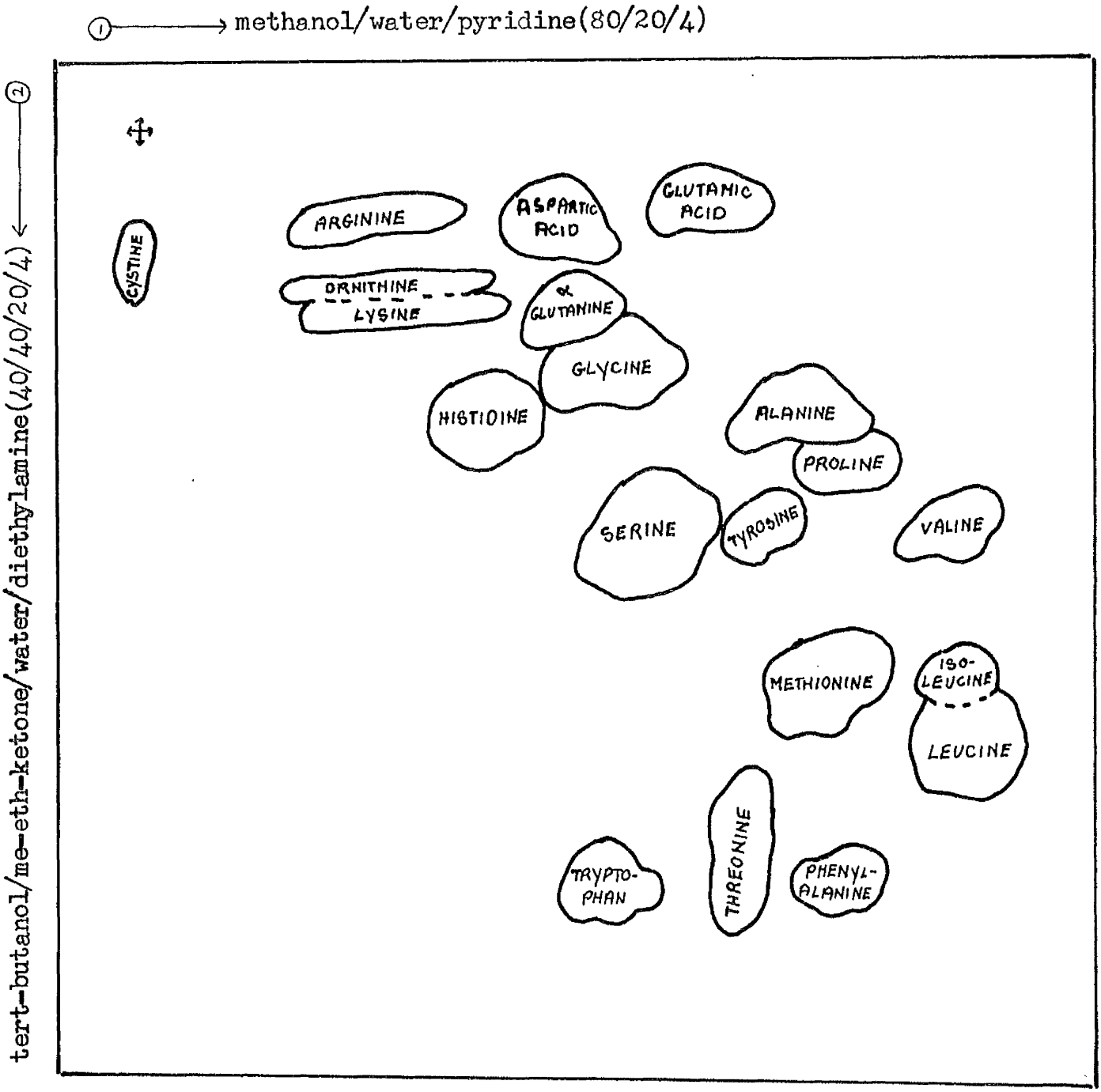


FIGURE 12 Tracing of chromatogram of standard mixture of amino acids run at room temperature - actual size.

examined for their free amino acid content (see Table 23); this proved very similar in most cases. Typical chromatograms for strain 524, D.kloeckeri, and strain 601, C.parapsilosis, are reproduced in Figures 13 and 14, respectively.

Glutamic acid, aspartic acid, serine, alanine, cystine, glycine, arginine, glutamine, lysine/ornithine, valine, threonine and iso-leucine/leucine were detected in every strain tested and only with histidine, tyrosine, phenylalanine and proline was any difference of occurrence apparent - see Table 23. Neither methionine nor tryptophan was detected in any of the strains and proline was found only in strain 314, C.parapsilosis. Little correlation between these differences and the species or original habitat of the organisms is apparent and until such time as a quantitative study is undertaken comparisons of minor differences may be misleading when only trace quantities are involved.

A spot which has been labelled "unknown X" was found to occur on the chromatograms of every strain and having a position near the original spot was thought to be perhaps a peptide or some peptide breakdown product.

Conclusion

In view therefore of the similar qualitative composition of the amino acid pools of these yeast strains no strain or even species differentiation on this basis seems possible but further work on a quantitative basis may prove more profitable in this regard.

TABLE 23

Occurrence of phenylalanine, histidine, tyrosine and proline in yeasts:-

Strain No.	Species	Phenyl-alanine	Histidine	Tyrosine	Proline
X524	D.kloeckeri	+	trace	trace	-
43	"	trace	"	-	-
157	"	"	+	-	-
7	"	-	-	-	-
301	"	trace	trace	-	-
249	C.lipolytica (var)	"	-	-	-
X180	T.inconspicua	"	-	-	-
150	"	"	trace	trace	-
134	T.inconspicua (var)	"	"	"	-
240	"	"	"	"	-
X601	C.parapsilosis	+	+	trace	-
2	"	trace	trace	-	-
314	"	+	"	trace	+
304	D.subglobosus	trace	-	-	-
X459	"	"	-	-	-
427r	Rh.mucilaginosa	"	-	-	-
443p	Rh.glutinis var.rubescens	-	-	-	-
501	M.krissii	-	-	-	-

X Strains of terrestrial origin.

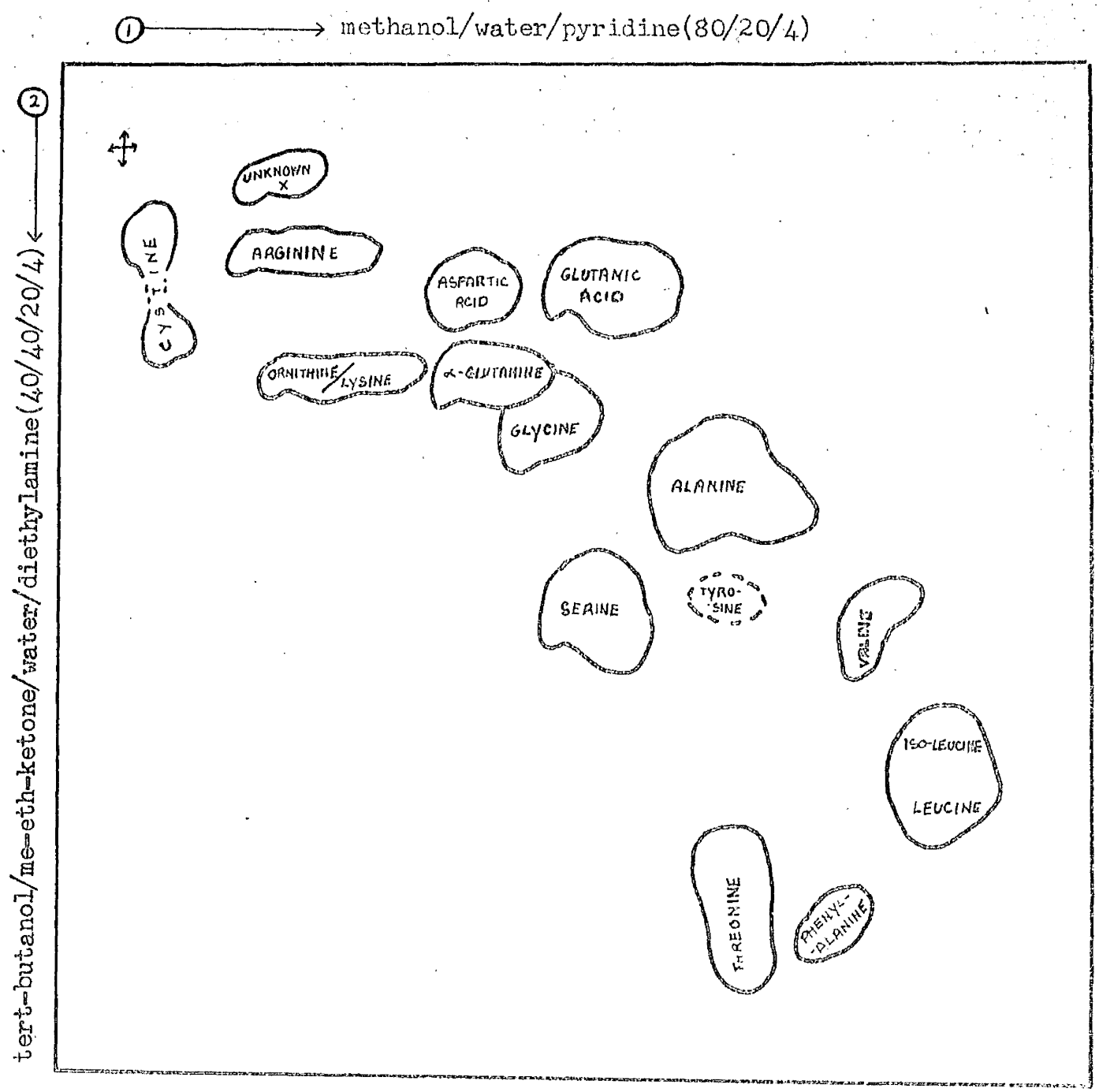


FIGURE 13 Tracing of chromatogram, 524, Debaryomyces kloeckeri, run at room temperature - actual size.

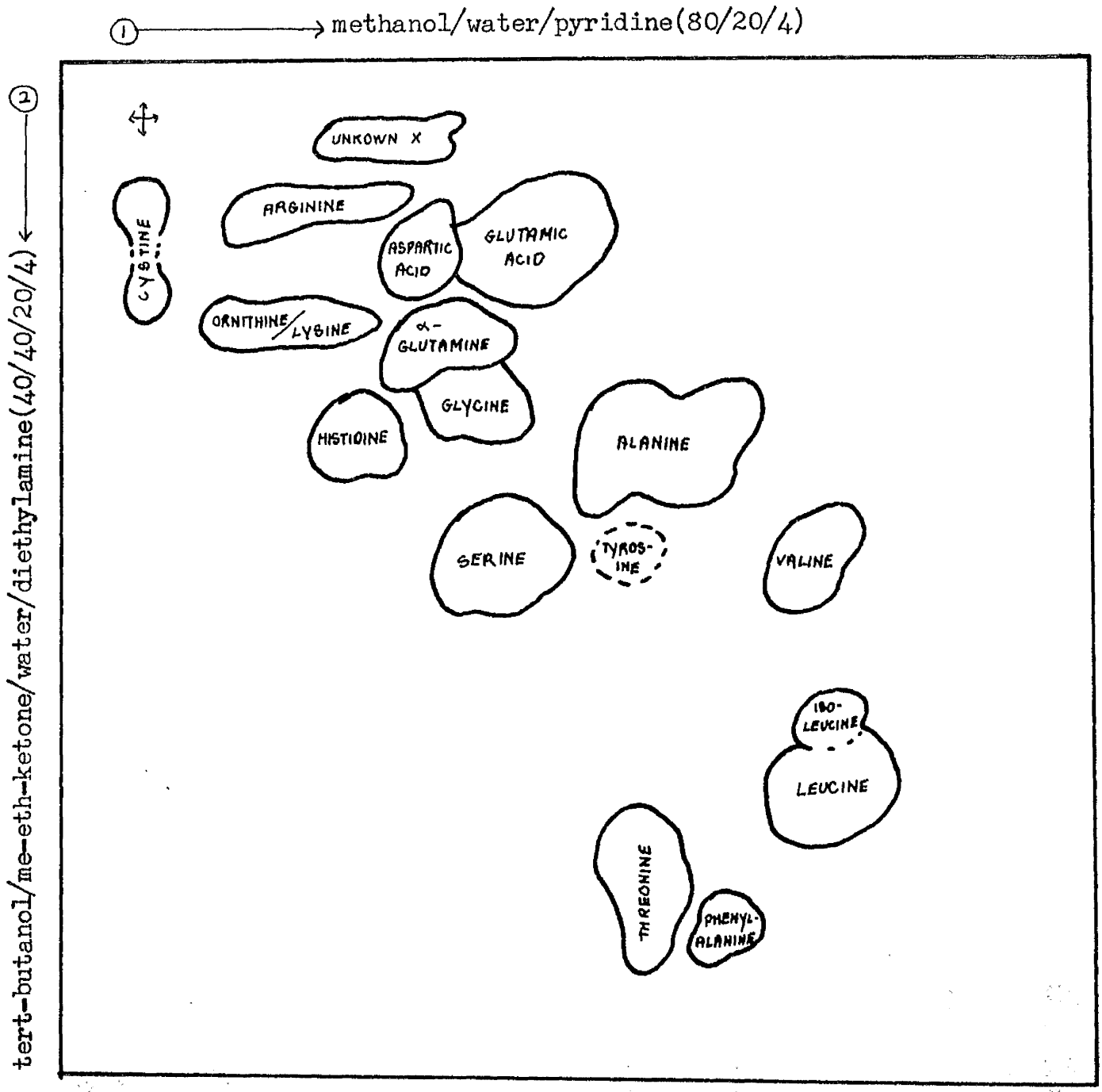


FIGURE 14 Tracing of chromatogram, 601, Candida parapsilosis, run at room temperature - actual size.

THE EFFECT OF SODIUM CHLORIDE ON GROWTH

Introduction

Salinity is an important ecological factor in the marine environment and the type of growth response produced by marine-occurring micro-organisms in varying concentrations of sodium chloride is frequently quoted as a taxonomic characteristic of these organisms.

Phaff et al. (1952) found while investigating the yeast flora of shrimps that some of their isolates were able to tolerate up to 16% w/v sodium chloride. Bhat et al. (1955) made similar findings amongst yeast isolates from the Indian Ocean, although some of them were able to grow in up to 21% w/v NaCl. Taken as a whole the group of isolates of the latter workers grew well in about twice the salt concentration (14 - 16% w/v) tolerated by corresponding terrestrial forms (7 - 9% w/v). The findings of these two groups of workers suggest that yeasts of marine origin are mainly euryhaline, i.e., capable of growing in a wide range of salinity, and hence in this study it was deemed necessary to employ a wide range of sodium chloride concentrations, i.e. 0.0 to 25.0% w/v, when estimating maximum salt tolerance.

It was also considered an essential part of this investigation to study the effect of sodium chloride on rate of growth and optimum cell yield as well as on ability to grow. Further, it was hoped to distinguish between "true" marine yeasts and yeasts of terrestrial origin/

origin by comparing the effect of NaCl on yeasts isolated during this survey and on those of terrestrial origin.

(a) Growth responses and maximum tolerance to various concentrations of sodium chloride

Methods:

Initially growth responses were determined using a basal medium of "MYGP" broth to aliquots of which were added various concentrations of sodium chloride. Portions of each solution (9.0 ml) were placed in optically matched tubes to which 1.0 ml of a standard cell suspension was added. After the cultures had been incubated unshaken for the required period of time growth was determined turbidimetrically in an absorptiometer. Reproduceability of results by this method was poor. It became apparent that this variation between experiments was caused by two main factors - slight differences in the composition and colour of the various batches of media and also the effects of incubation in a static position resulting in poor mixing of the culture and limitation of the oxygen supply.

These faults were overcome by agitating the cultures and by using the following colourless, almost synthetic, basal medium - medium "B":-

Medium "B"/

Medium "B"

$(NH_4)_2SO_4$	-	0.5% w/v
KH_2PO_4	-	0.1% w/v
$MgSO_4.7H_2O$	-	0.05% w/v
$CaCl_2.2H_2O$	-	0.01% w/v
(A/R) Glucose	-	1.00% w/v
* Yeast water	-	2.5% v/v
NaCl	-	as required
De-ionised water to		100%

pH adjusted to 5.0

* (Yeast extract 0.5% w/v in water)

Each medium held in 20 ml portions in 50 ml conical flasks was inoculated with a suspension of cells (1.0 ml) containing approximately 6 x 10^6 cells/ml prepared from a three-day-old culture grown on "MYGPS" agar at 25°C. These cultures were then incubated at 25°C on a shaker with an amplitude of 7.5 cm., at 60 cycles/min. Samples were removed from each flask after 18, 24, 42 and 48 hours and the amount of growth estimated turbidimetrically in an Eel absorptiometer using a neutral density filter and water as a blank. Optical density readings were related to the number of cells/ml by means of a calibration curve.

Cultures showing no measureable growth after 48 hours were left/

TABLE 24

The highest concentration of sodium chloride in which growth occurred at 25°C and time when such growth was observed:-

Species and Strain No.	Highest % w/v NaCl allowing growth	Time for visible Turbidity to appear - in days
405 D.subglobosus	22.0	17
325 "	20.0	13
* NCYC 459 "	18.0	6
302 D.kloeckeri	23.0	25
354s "	23.0	18
281 "	22.0	10
132 "	22.0	19
246s "	22.0	8
169 "	22.0	14
43 "	22.0	13
* NCYC 8 "	20.0	21
67 T.famata	19.0	13
* NCYC 1 "	18.0	10
189 P.membranaefaciens	22.0	15
* NCYC 54 "	14.0	18
321p Rh.glutinis var.rubescens	22.0	8
248p " "	18.0	10
* RCST 92 Rh.glutinis	14.0	13
501 M.krissii	19.0	10
62 C.parapsilosis	18.0	13
309w "	18.0	10
158 "	18.0	7
* NCYC 601 "	19.0	10
150 T.inconspicua	15.0	8
* CBS 180 "	9.0	14
318 T.inconspicua (var.)	11.0	10
115 " "	11.0	12
238 C.lipolytica (var.)	14.0	19
354L " "	14.0	21
* RCST 144 S.carlsbergensis	11.0	18
BY1 P.pullulans.	9.0	8

* Strains of terrestrial origin.

RCST Royal College of Science and Technology
NCYC National Collection of Yeast Cultures
CBS Centraalbureau voor Schimmelcultures

left in the incubator and the time noted when subsequent growth appeared. Incubation was stopped after four weeks and the highest concentration of NaCl affording growth during this period was taken as the maximum salt tolerance. Strains tested by this procedure are indicated in Table 24. A number of strains were tested only for maximum salt tolerance and are also indicated in Table 24.

Results:

All the strains of Debaryomyces kloeckeri, including strain 8, the NCYC strain, gave a similar growth pattern in which the maximum yield of cells after 48 hours occurred in 1.0 - 3.0% w/v NaCl. Strains 246s, 281, 169, 43 and 302 (see Figure 15) exhibited this growth peak in 2.0% w/v NaCl; 354s and 8 (see Figure 16) in 1.0% w/v NaCl and 132 in 3.0% w/v NaCl. Maximum cell production by any of these cultures never exceeded 6×10^8 cells/ml.

Strains 150, 321p, 62, 189 (see Figure 17), 238, 144 and 54, which represent other yeast species, exhibited a different growth pattern in which maximum growth occurred in the medium containing no sodium chloride. A sharp fall in cell production occurred with increasing concentrations of sodium chloride. The maximum yield of cells for all this group of yeasts, except strains 150 and 144, was significantly higher than that attained by Debaryomyces kloeckeri.

The time for visible turbidity to appear in the higher concentrations/

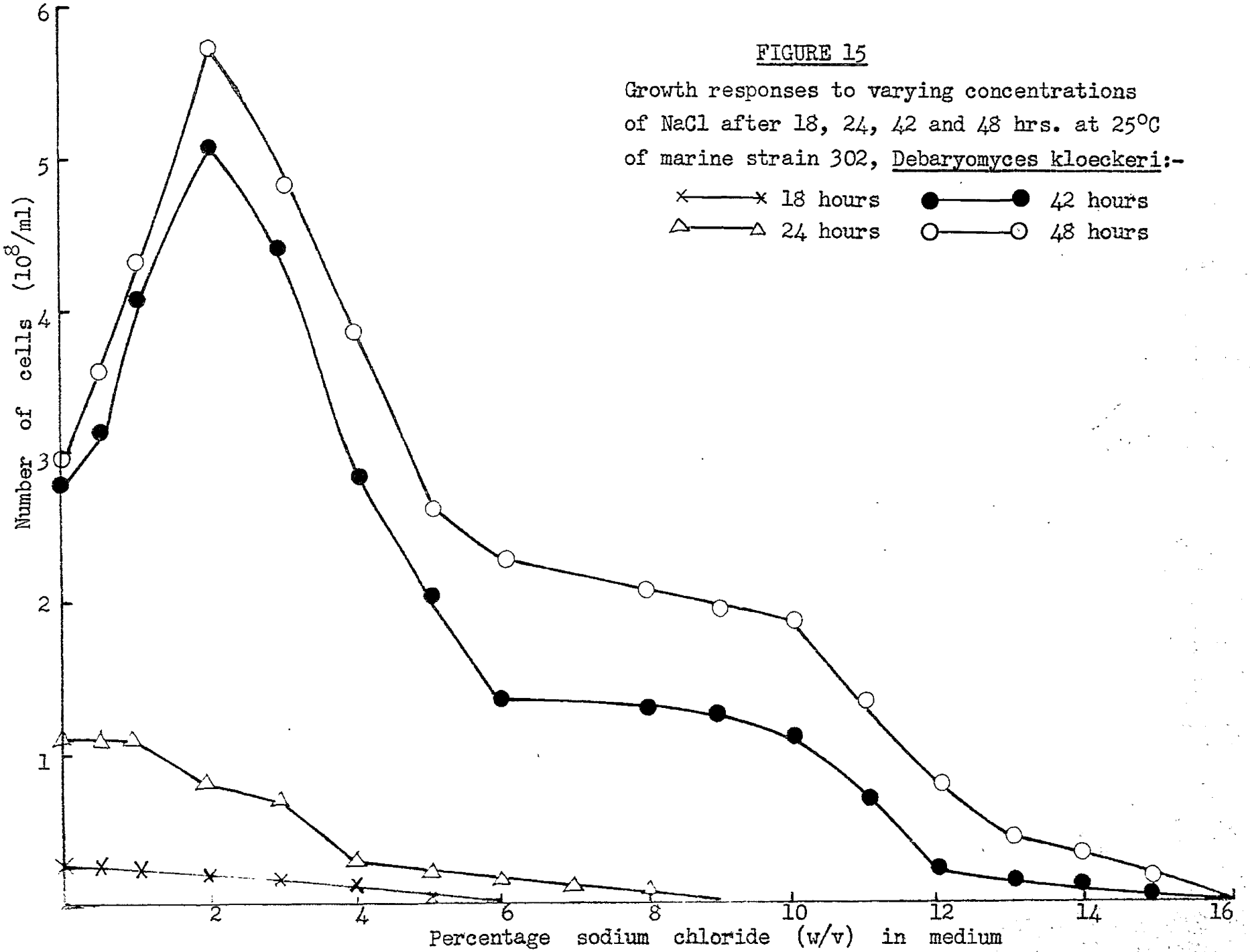


FIGURE 15

Growth responses to varying concentrations of NaCl after 18, 24, 42 and 48 hrs. at 25°C of marine strain 302, Debaryomyces kloeckeri:-

FIGURE 16

Growth responses to various concentrations of NaCl after 18, 24, 42 and 48 hrs. at 25°C of NCYC strain 8, Debaryomyces kloeckeri:-

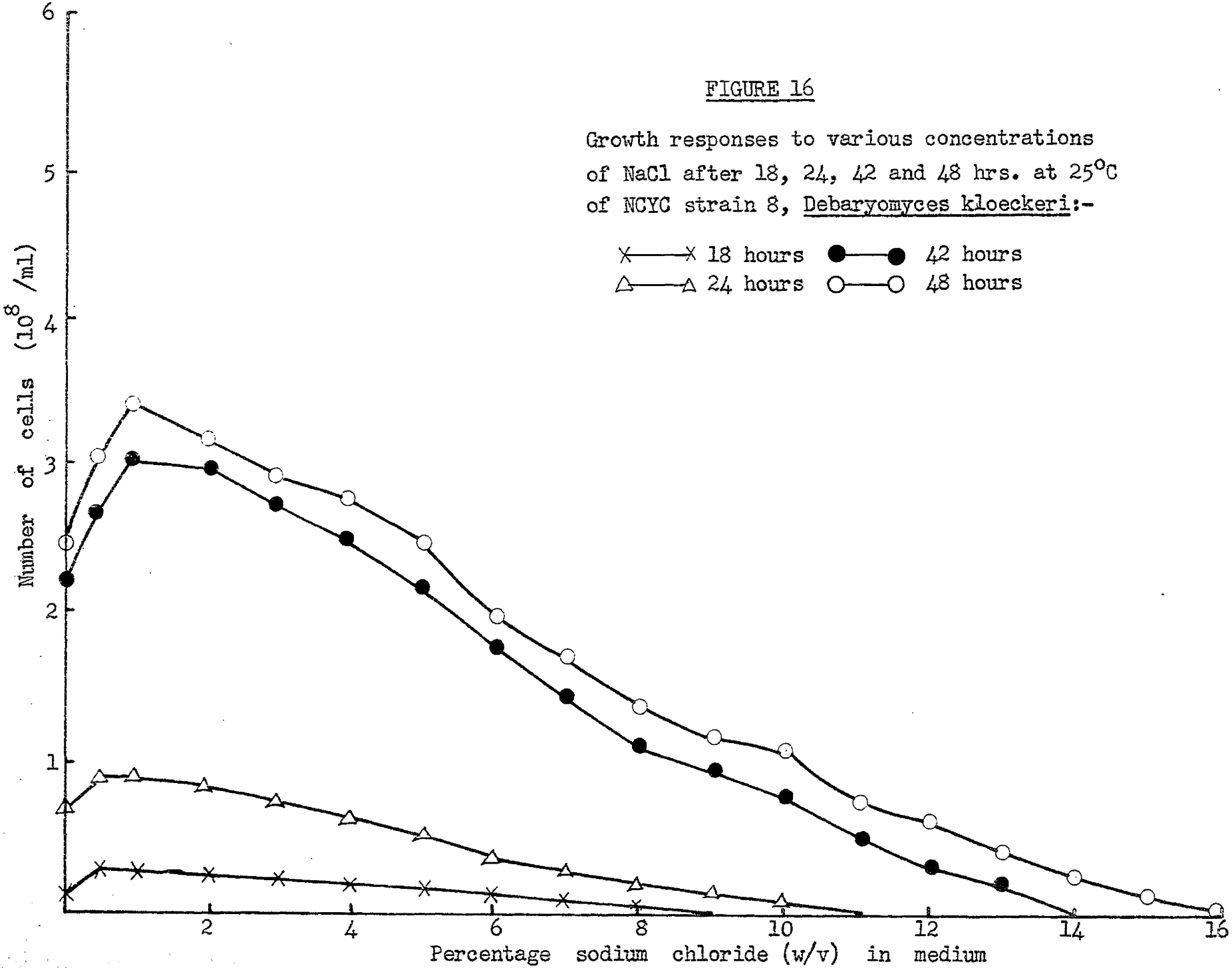
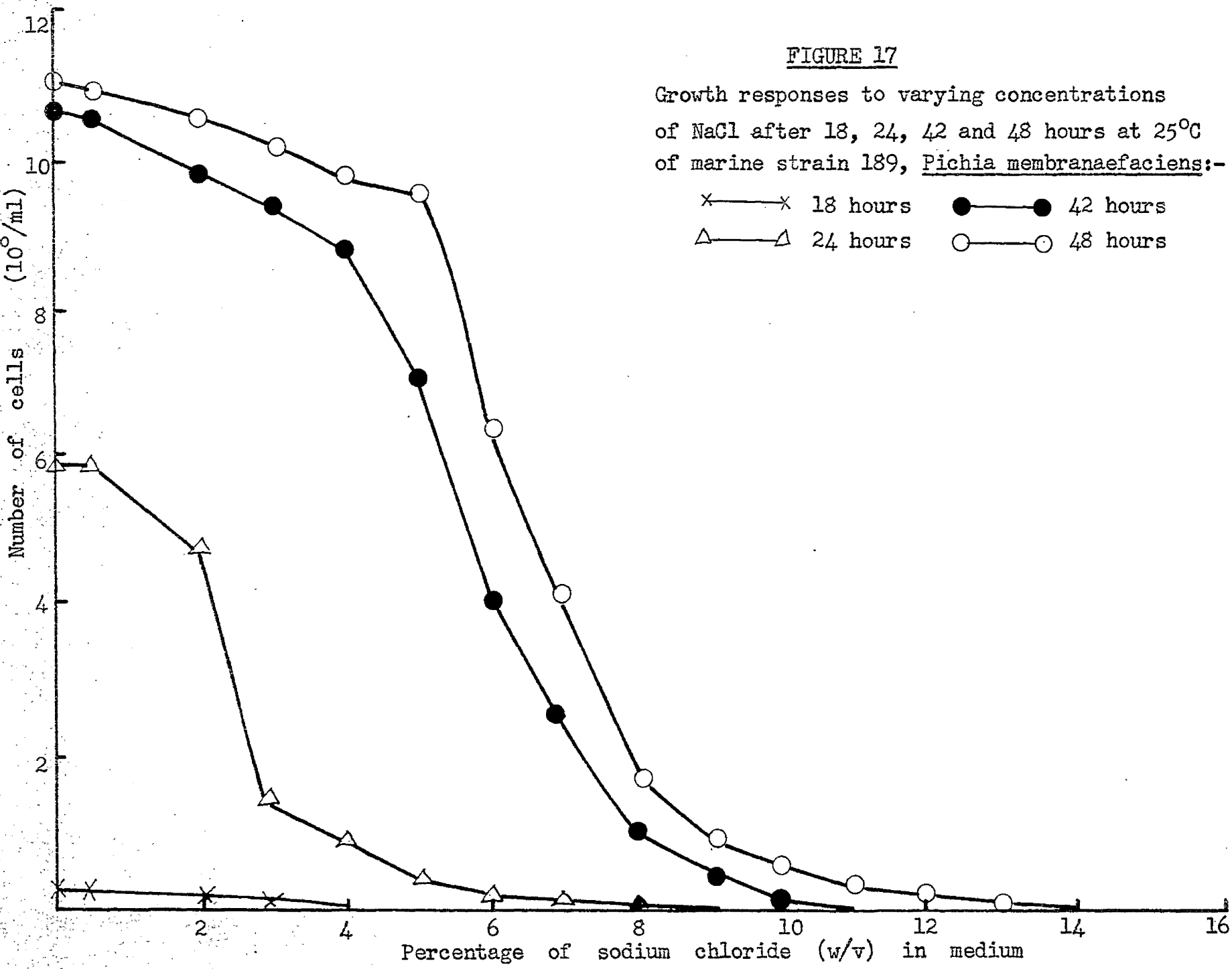


FIGURE 17

Growth responses to varying concentrations of NaCl after 18, 24, 42 and 48 hours at 25°C of marine strain 189, Pichia membranaefaciens:-



concentrations of sodium chloride is noted in Table 24. The highest halo-tolerance was exhibited by all the strains of Debaryomyces kloeckeri. D. subglobosus and by strain 189, Pichia membranaefaciens and strain 321p, Rhodotorula glutinis var.rubescens. Good production of pink pigment was maintained by the last-mentioned strain in these high salt concentrations. The time lapse before growth appeared at the points of maximum tolerance varied greatly among the strains and no correlation between time and concentration is obvious. It was quite noticeable that no matter how long the lag phase, as soon as growth became obvious the subsequent growth was very rapid.

(b) Growth rates in varying concentrations of sodium chloride

Introduction:

In view of the above-mentioned differences in growth response between the strains of D. kloeckeri and the other strains tested and the fact that rapid growth always followed a lag period in the higher salt concentrations, which permitted growth of certain strains, it was decided to investigate the growth rates of strains 43, D.kloeckeri and 189, P.membranaefaciens, in varying concentrations of sodium chloride.

Methods:

Two series of three tubes with 70 ml of medium "B", containing 1.0%, 4.0% and 8.0% w/v NaCl, respectively, were sterilised. One series/

series was inoculated with 1.0 ml of a standard cell suspension of yeast strain 43 and the other series similarly with strain 189. The cultures were incubated at 25°C and mixed by a constant stream of sterile air. Samples were removed at frequent intervals during a period of approximately ninety hours and the number of cells/ml estimated as in (a).

Results:

The growth curves obtained for strain 43, D.kloeckeri, grown in the presence of 1.0%, 4.0% and 8.0% w/v NaCl indicate that in these lower salt concentrations the rate of growth during the exponential phase was unaffected but the duration of this logarithmic growth was decreased slightly in 4.0% and quite significantly in 8.0% w/v NaCl. Measurements of optical density below the value corresponding to 1×10^6 cells/ml are not possible using the Eel absorptiometer but by extrapolation of the growth curves to a base line, representing the rate of inoculation, a slight increase in the lag phase from 1.0 - 4.0% was obvious and a much larger increase was discerned from 1.0 - 8.0%, (see Figure 18). The growth curves of strain 189, P.membranaefaciens, obtained under similar conditions exhibit a similar pattern to those of strain 43 except that a greater prolongation of the lag phase in 8.0% was shown by the former strain (see Figure 19).

These experiments also revealed that strain 189 gave a higher cell yield in 1.0 and 4.0% w/v NaCl (11.8×10^8 and 8.8×10^8 cells/ml, respectively)/

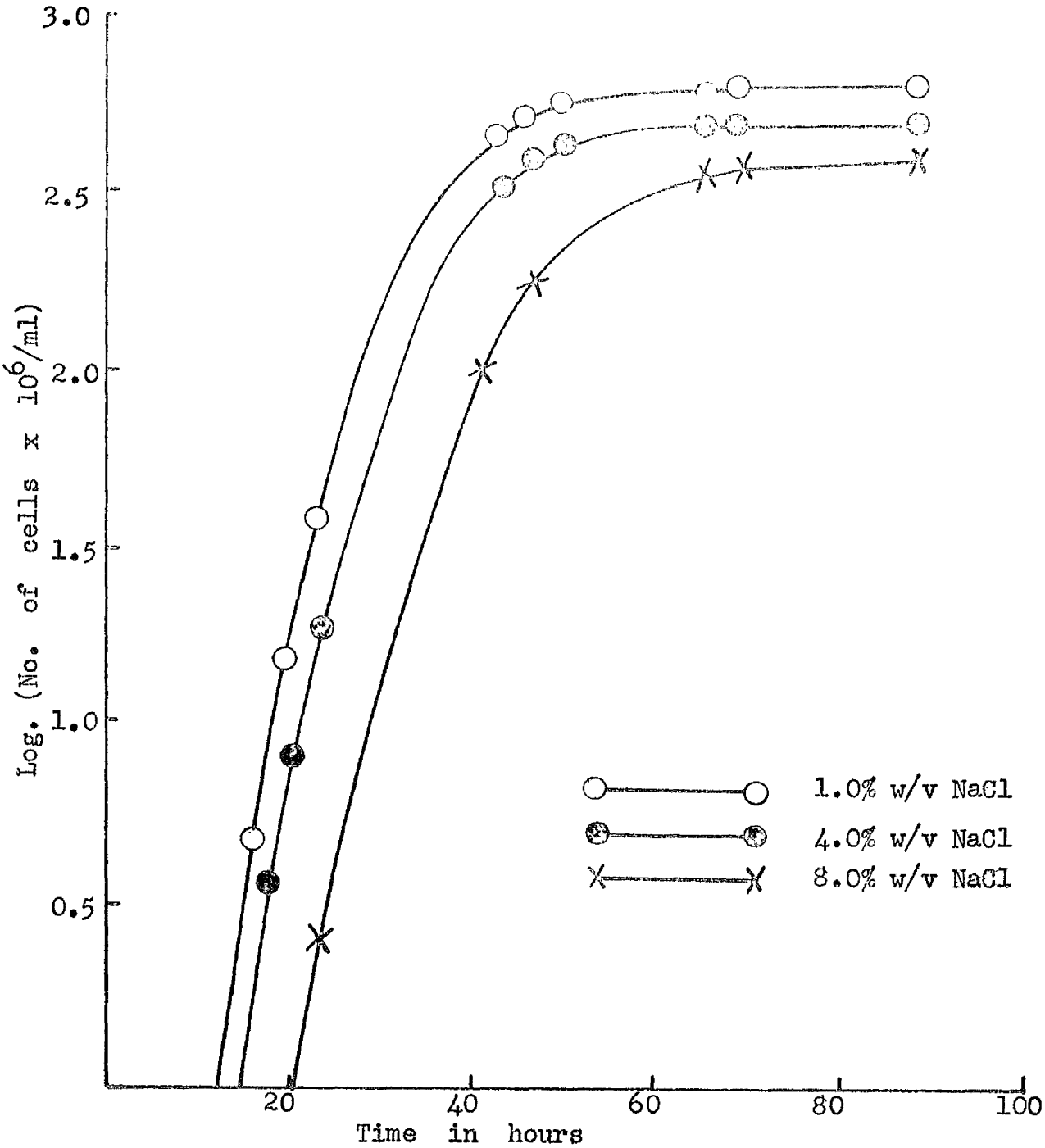


FIGURE 18 Growth curves of strain 43, D.kloeckeri, in media containing 1.0, 4.0 and 8.0% w/v NaCl, at 25°C.

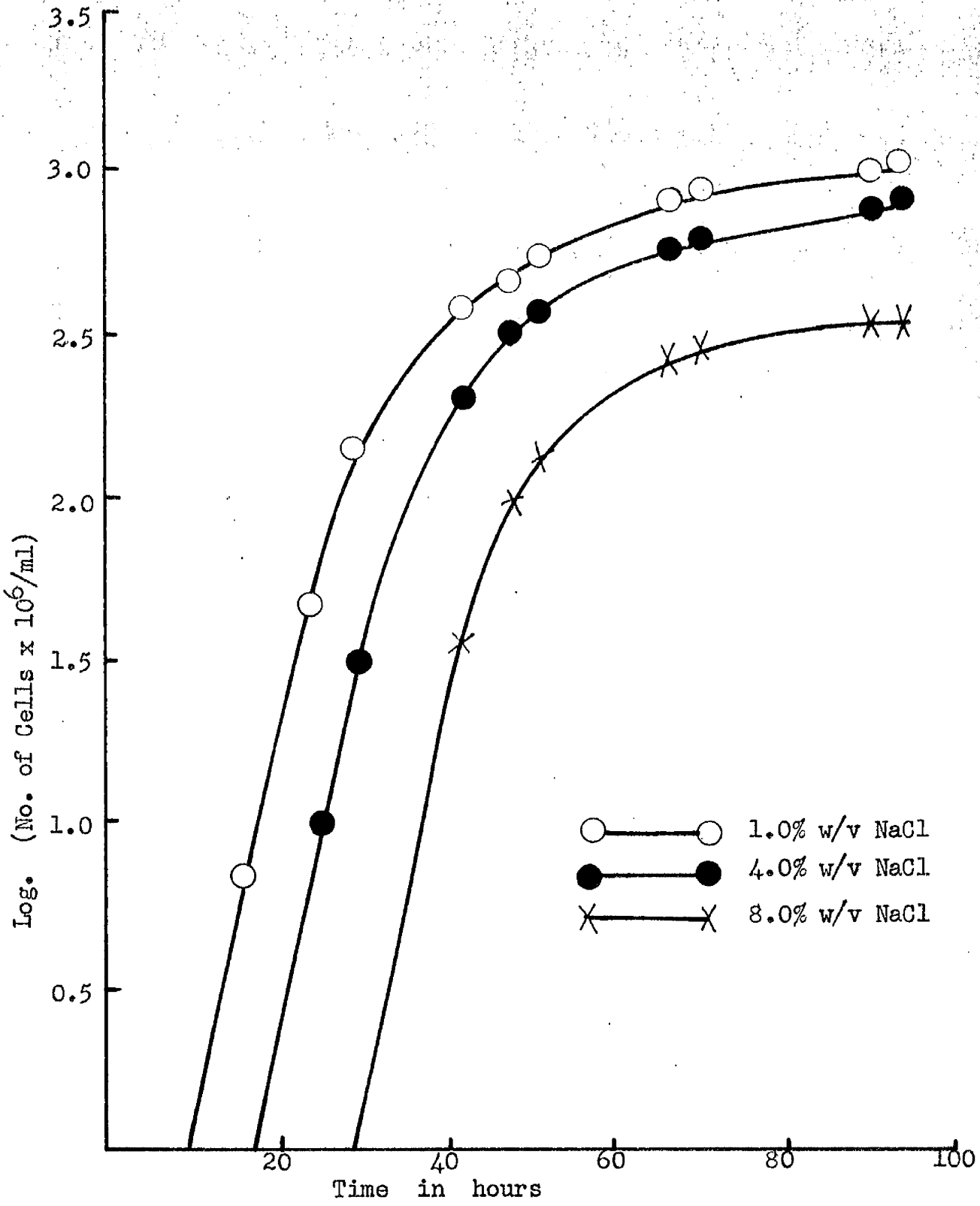


FIGURE 19 Growth curves of strain 189, P.membranaefaciens, in media containing, 1.0, 4.0 and 8.0% w/v NaCl, at 25°C.

ml, respectively) than did strain 43 (7.2×10^8 and 5.6×10^8 cells/ml, respectively). However, in 8.0% w/v NaCl the maximum cell yield of strain 43 (4.6×10^8 cells/ml) was higher than that for strain 189 (3.6×10^8 cells/ml).

(c) Effect of the nitrogen source on the rate of growth in sodium chloride and on maximum halo-tolerance

Introduction:

During the present studies preliminary work showed that it was advantageous to use simple media with ammonium sulphate as the notrogen source. However, it was appreciated that this might provide a somewhat different set of conditions from that prevailing in marine waters. Consequently it was considered advisable to ascertain whether the results could be influenced by the presence of more complex sources of nitrogen.

Methods:

(i) Effect on growth rate:

A batch of double-strength medium "B" containing 10% w/v NaCl but minus ammonium sulphate was prepared. This medium was divided into five parts each of which received a different nitrogen source in amounts calculated to give 0.0212% g nitrogen in the final single-strength media. The sources of nitrogen were urea, asparagine, peptone, ammonium sulphate and fish extract. The last-mentioned source/

source was an aqueous extract prepared by macerating filleted whiting in a Waring blender, filtering and using the filtrate. The media were made up to volume to give single-strength media and their pH adjusted to 5.0 before autoclaving. Three 70 ml aliquots of media containing ammonium sulphate, urea and asparagine, respectively, were placed in sterile tubes which were then inoculated with 1.0 ml of a standard cell suspension of strain 43, D.kloeckeri. The cultures were then incubated and the readings taken as in (b). The experiment was repeated with the same yeast but using media containing ammonium sulphate, peptone and fish extract, respectively.

(ii) Effects on maximum halo-tolerance:

Five batches of medium "B" were prepared as for the previous test except that aliquots of each batch of medium contained 21.0, 22.0, 23.0 and 24.0% w/v NaCl, respectively. Portions of each solution (20 ml) were placed in 50 ml conical flasks which were plugged and sterilised. The flasks were inoculated with 1.0 ml of standard cell suspensions prepared from yeast strains 189, 354s, 132 and 321p. The flasks were incubated at 25°C on the shaker for six weeks and the times at which the cultures showed growth were noted.

Results:

The effects of varying the nitrogen source on the growth of strain 43, D.kloeckeri, grown in the presence of 5.0% w/v NaCl appear similar to those caused by varying the NaCl concentration when the nitrogen source was ammonium sulphate as in the series above. The growth/

growth rate during the exponential phase was unaffected but the duration of this phase varied greatly with the nitrogen source. Also, the shorter the exponential phase the smaller was the maximum cell yield and the longer the apparent lag phase.

In one set of experiments asparagine appears to allow better growth than ammonium sulphate which is itself better than urea (see Figure 20). In another set of experiments ammonium sulphate proved to be a poorer nitrogen source than fish extract, while peptone was the best (see Figure 21).

Discrepancies between the results for ammonium sulphate in the two experiments was most likely due to differences in the rate of aeration in the two tests. From these investigations it can be concluded that shorter lag phases and higher cell yields occur with peptone, fish extract and asparagine than with ammonium sulphate and urea.

The time required for growth to occur, if any, during six weeks' incubation with 21.0, 22.0, 23.0 and 24.0% w/v NaCl, using the five different nitrogen sources with yeast strains 189, 354s, 132 and 321p is given in Table 25. The four yeasts grown in ammonium sulphate medium exhibited the same halo-tolerance as found in previous tests and listed in Table 24. There is, however, some difference in the period of time taken to reach these points of maximum tolerance on the/

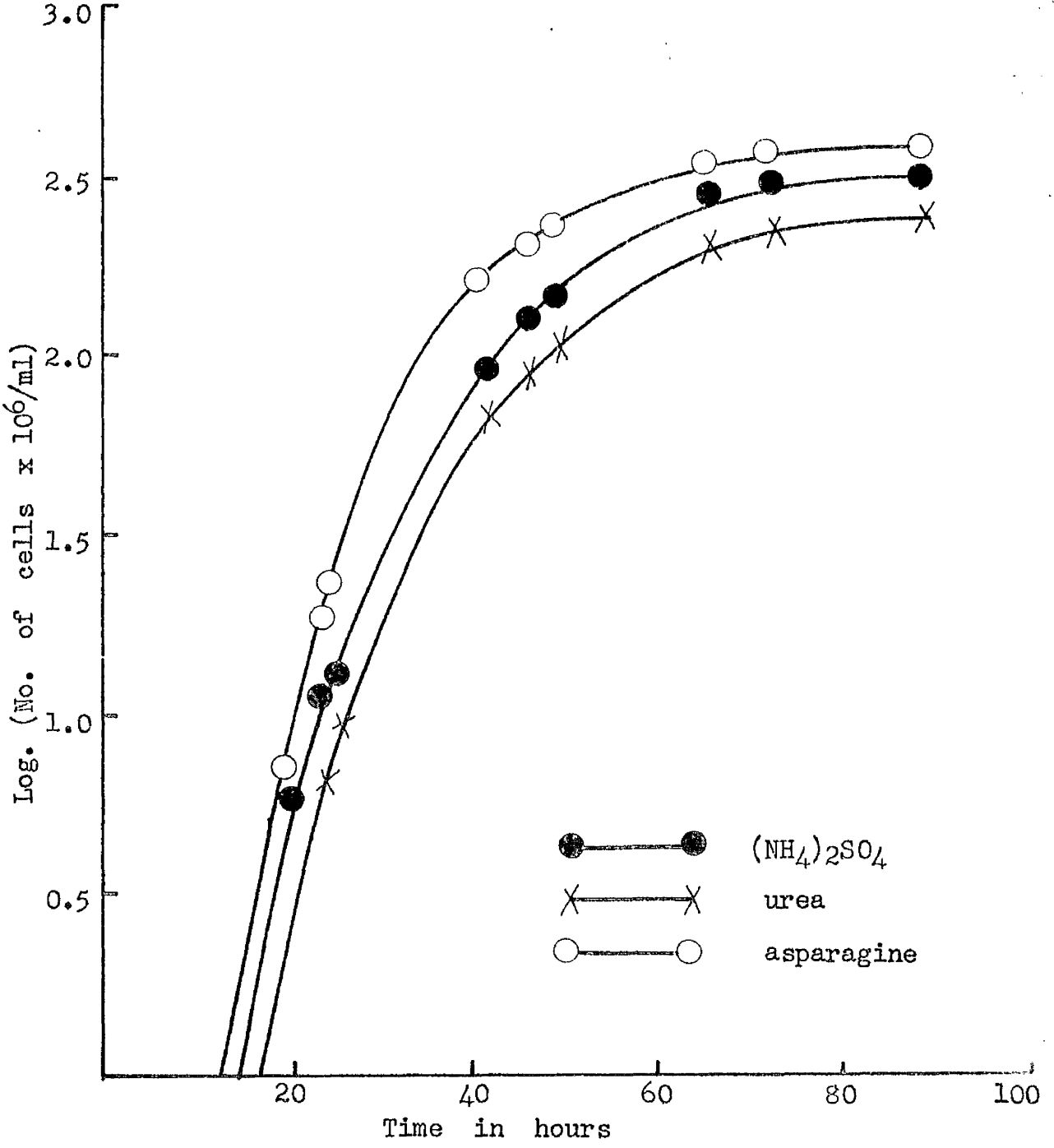


FIGURE 20 Growth curves of strain 43, Debaryomyces kloeckeri, in 5.0% w/v NaCl, using asparagine, urea and ammonium sulphate, at 25°C.

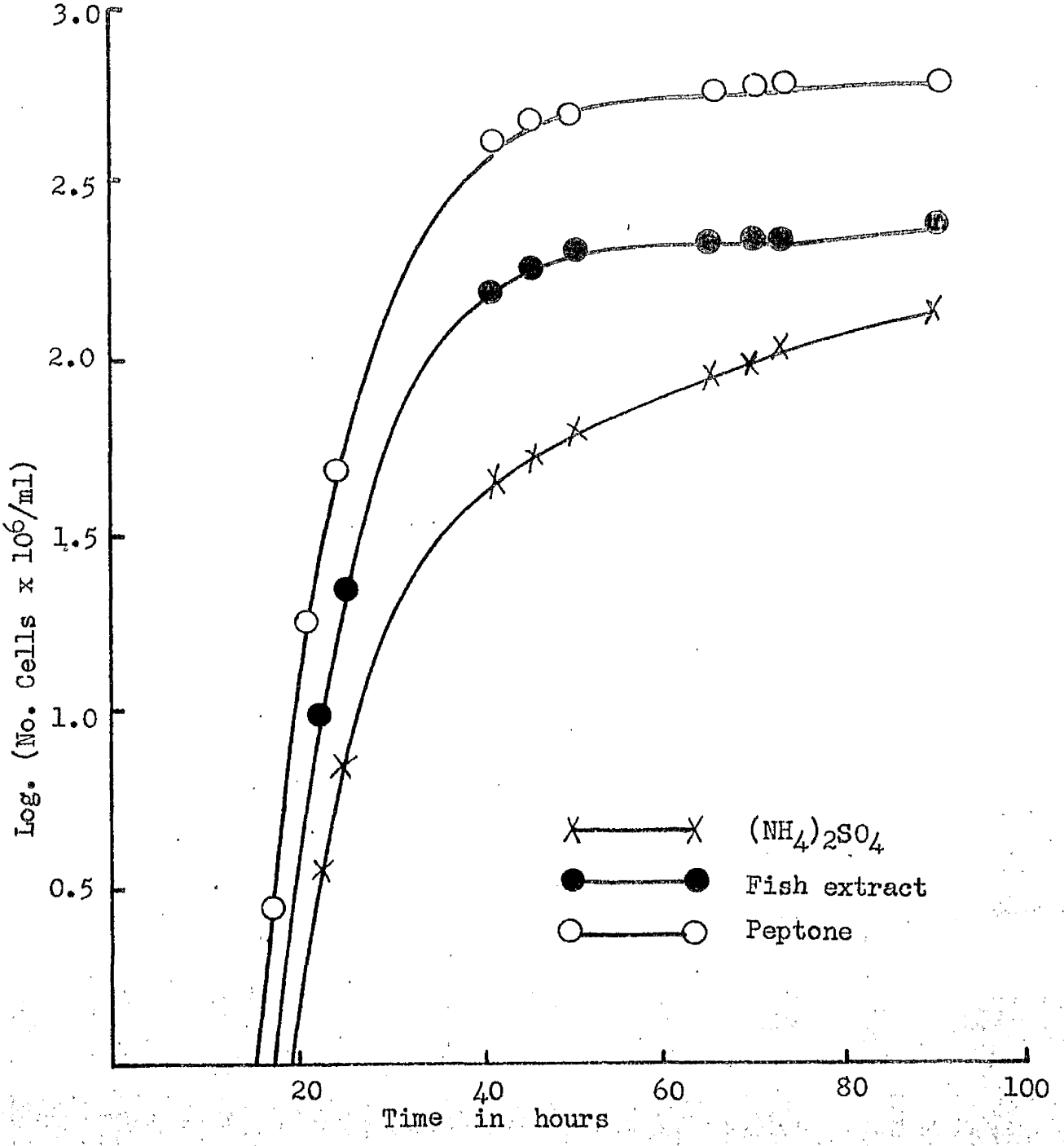


FIGURE 21 Growth curves of strain 43, D.kloeckeri, in 5.0% w/v NaCl, using peptone, fish extract, ammonium sulphate, at 25°C.

the two occasions of testing. It could be postulated here that the ability of these yeasts to grow in a certain concentration of NaCl under standard conditions is a stable characteristic of these yeasts, although the time taken to initiate growth may be subject to small environmental or inoculum changes.

The results given in Table 25 indicate that this ability to tolerate a certain concentration of NaCl can be affected by the nature of the nitrogen source. Fish extract caused a significant increase in tolerance from that found using ammonium sulphate, whereas urea caused a decrease in tolerance. Although asparagine and peptone allowed quicker growth to occur they proved no better and in some cases poorer sources of nitrogen than ammonium sulphate in these high salt concentrations.

(d) The effect of storage on the maximum halo-tolerance

Introduction:

Results obtained by Mrak and Bonar (1939) from a study of film yeasts from pickle brines suggested that a gradual decrease in the salt tolerance of the micro-organisms occurred after two years' storage on wort agar slopes. This may explain the somewhat lower values of maximum halo-tolerance of terrestrial strains than those of the corresponding marine strains which were found by Bhat et al. (1955) and also in this present study (see Table 24) as these former strains/

TABLE 25

Maximum halo-tolerance in five different nitrogen sources:-

Species and Strain number	% w/v NaCl in medium	Days required for growth in				
		$(NH_4)_2SO_4$	Peptone	Aspara-:gine	Urea	Fish extract
P.membranaefaciens 189	21.0	21	6	10	-	9
	22.0	32	-	21	-	12
	23.0	-	-	-	-	26
	24.0	-	-	-	-	28
D.kloeckeri 354s	21.0	6	5	6	9	6
	22.0	10	7	9	-	7
	23.0	38	-	17	-	12
	24.0	-	-	-	-	21
D.kloeckeri 132	21.0	10	5	6	13	8
	22.0	21	21	-	-	10
	23.0	-	-	-	-	18
	24.0	-	-	-	-	28
Rh.glutinis 321p v.rubescens	21.0	11	10	9	24	10
	22.0	21	-	-	-	20
	23.0	-	-	-	-	32
	24.0	-	-	-	-	-

N.B. Blank spaces indicate that no growth occurred during six weeks' incubation.

strains had presumably, previous to testing, undergone a long period of laboratory cultivation. It was therefore decided to investigate the effects on the maximum halo-tolerance of certain yeast strains of a year's storage on media containing varying salt concentrations.

Results:

Strains 43, 132, 321p, 62 and 150 were maintained for one year with monthly subculturing on "MYGPS" agar and "MYGP" ager containing 4.0% and 8.0% w/v NaCl, respectively. Tests for maximum salt tolerance were then made as in (a) and the results are indicated in Table 26.

TABLE 26

Maximum halo-tolerance of yeast strains cultivated on media containing varying concentrations of NaCl for twelve months:-

Strain No.	Species	Initial maximum halo-tolerance	Maximum halo-tolerance after twelve months' storage on:- MYGPS	MYGP	MYGP + 4% NaCl	MYGP + 8%NaCl
43	D.kloeckeri	22.0	22.0	22.0	22.0	22.0
132	"	22.0	22.0	22.0	21.0	20.0
321p	Rh.glutinis var. rubescens	22.0	21.0	19.0	21.0	----
62	C.parapsilosis	18.0	18.0	18.0	17.0	16.0
150	T.inconspicua	15.0	14.0	14.0	14.0	14.0

As can be seen, little or no change was found in the maximum halo-tolerance/

halo-tolerance of the organisms cultured on "MYGPS" and apart from 321p which failed to survive on "MYGP" agar containing 8% w/v NaCl and exhibited a somewhat lower tolerance after cultivation on plain "MYGP" agar, there also appeared to be little change in the halo-tolerance of the micro-organisms cultivated on the other three media. Strains 132 and 62, however, did exhibit slightly decreased halo-tolerance with increased salt concentration in "MYGP" medium.

Conclusion

Figures published by Mrak and Bonar (1939) and Phaff et al. (1952) indicate that the time required for visible growth of yeasts to appear increases as the concentration of NaCl in the medium is raised. The present investigation has substantiated these results and revealed further that as the concentration of NaCl in the medium is increased to 8% w/v so the lag phase increases and the duration of the exponential phase decreases while the rate of growth shows little or no change. The extent of these changes may vary according to the strain being studied.

The results also indicate that the maximum concentration of salt tolerated by these yeasts is a feature of the strain and that there is a correlation between the halo-tolerance of particular species isolated from different marine locations. Similar results

were/

were reported in the case of yeasts isolated from shrimps by Phaff et al. (1952) and from food brines by Mrak and Bonar (1939), Drake et al. (1959) and Etchells et al.(1961).

It can be seen from the above results that although a particular strain will always tolerate tha same order of salt concentration the time required to achieve this may vary: this is difficult to explain. One possible explanation is that the culture represents a heterogeneous population, all cells of which possess the characteristic necessary to place it in a specific rank but differ in other details, and the high concentration of salt exerts some form of selective action. This type of culture is not unknown. For example, single cell isolates from an industrial strain of yeast may be shown to differ in their individual rates of fermentation but a fairly constant amount of end-product is obtained eventually from most of the isolates (Morris, personal communication).

At present there is still relatively little known about the physiological changes taking place in the cells during the lag phase. Hence it is difficult to explain the increase in duration of this phase as the concentration of salt in the medium is increased. Perusal of the literature does, however, indicate a possible explanation in broad terms. Thus Takada (1956) and Tolberg and Pace (1960) showed that the concentration of Na^+ ions increased within the yeast cell as the concentration of NaCl is raised in the growth media. Ingram (1938) also/

also found that the internal concentration of Na^+ ions in cells of Saccharomyces cerevisiae in various salt solutions was of the same order as that of the external medium over a range of 1-2 M. The results of Takada (1956) and Tolberg and Pace (1960) further indicate that as the concentration of NaCl is increased in the medium so the K^+ ions in the cell - both free and bound - are replaced, at least to some extent, by Na^+ ions. Onishi (1959) also found that cells cultivated with high concnetrations of NaCl contained much less K^+ ions than those grown without NaCl and Conway and Moore (1952) have described the production of cells of Saccharomyces cerevisiae in which 98% of the intracellular K^+ ions were replaced by Na^+ ions i.e., "sodium" yeasts; "ammonium" yeasts were produced similarly. It seems probable then that in increased concentrations of NaCl, Na^+ ions somehow penetrate the cell membrane and replace K^+ and other ions which, from the evidence of Tolberg and Pace (1960), Takado (1956) and Takado and Takuno (1958), may exist in different cellular compartments.

Ingram (1957) has suggested that halophilic bacteria survive in high concentrations of salt because they possess enzymes adapted to high intracellular salt concentrations and the same may also be true of these halo-tolerant yeasts, which, as the evidence suggests, are poikilosmotic, i.e., intracellular osmotic pressure can vary over a wide range, depending on the osmotic pressure of the medium (Tolberg and Pace, 1960). Thus the increased lag phase is probably due/

due to one or a combination of the following factors - the time for preformed enzymes to become adapted to tolerate high salt concentra-:tions; the time for new tolerant enzymes to be formed; the time for the development of enzymes in which Na^+ replaces K^+.

The stimulatory effect of complex organic nitrogen sources on the growth of the yeasts has been demonstrated and similar effects have also been noted with halophilic bacteria by Katznelson and Lochhead (1952), Castell and Mappleback (1952) and Dussault and Lachance (1952). It has also been shown that these materials increase the halo-tolerance of yeasts. Thus, it would appear likely that high concentrations of NaCl interfere with the metabolic pathways concerned with the conversion of simple sources of nitrogen to internal amino-acids. This hypothesis is supported by the observations of Nagai (1953) who found less extractable amino-acids in yeast strains showing poor growth in media containing higher concentrations of NaCl.

The halo-tolerance of both marine yeasts and of certain terrestrial yeasts which so far as can be established had no previous history of contact with salt, indicate that many strains of yeast are able to tolerate high concentrations of salt. The range of yeasts isolated from food brines further illustrates this conclusion - Joslyn (1927), Joslyn and Cruess (1929/30), Hof (1935), Mrak and Bonar (1938) (1939), Graham and Hastings (1942), Etchells and Bell (1950), Zenitani (1952), Etchells et al. (1953), Costilow et al. (1954), Drake et al. (1959)/

(1959) Etchells et al. (1961). Furthermore, it is interesting to note that in many instances the pattern of yeast species obtained from such brines is similar to that obtained from marine sources during this survey.

Although high salt tolerence may not be indicative of a "true" marine yeast the fact that the optimum concentration of NaCl for the growth of strains of Debaryomyces kloeckeri is approximately in the same order as that in the seas, could be of ecological significance. Similar salt preferences have been exhibited by some marine algae and fungi - Vaisey (1954), Ritchie (1959), Provasoli (1958), Siepmann (1959), Vishniac (1960), McLachlan (1961) - although they have much narrower limits of tolerance than these yeasts. However, the fact that the terrestrial strain of D.kloeckeri behaved in a similar manner to the marine isolates and that recently Merdinger and Shair (1962) found strains of Debaryomyces hansenii isolated from a terrestrial habitat to exhibit optimum growth in media containing 2-3% w/v NaCl as well as tolerating 17-18% w/v NaCl, does make it difficult to uphold the contention that the strains of D.kloeckeri are "true" marine yeasts because they exhibit optimum growth in media containing a salt concentration similar to that of the seas. Further it is known that D.kloeckeri is commonly isolated from food brines in inland areas far from the sea.

The findings described here, however, do illustrate that these yeasts/

yeasts are capable of surviving in both fresh-water and in marine salt-water if other growth conditions are favourable, e.g.,temperature, food, pH, etc. In this connection it is also interesting to note that one of the strains of D.kloeckeri, 281, which was isolated from a salmon, returning from the sea, fourteen miles up the River Dee, exhibited the same salt tolerance and other characteristics as the marine isolates and might probably have been carried by the fish from the sea. However, it does seem that growth response to varying concentrations of salt will not alone serve to indicate the true ecological niche of these marine isolates, even those of D.kloeckeri.

It is well established that marine teleosts maintain a lower salt concentration in their body fluids than that of the surrounding sea-water and to prevent dehydration they are continually swallowing sea-water. The salt ingested with this sea-water, however, must be excreted and as no great quantity of salt has been detected in either the urine or faeces the existence of "chloride-secreting" cells has been postulated (Baldwin, 1949). This hypothesis has not yet been confirmed or rejected and only a little evidence has accumulated to date suggesting that these cells may exist in the respiratory epithelium of the gills (Brown, 1951). If such cells do exist, however, they may be an important ecological factor as the micro-environment surrounding them will have a higher salt concentration than the sea-water and only a micro-flora capable of tolerating these conditions will survive at these sites.

OCCURRENCE AND SPECIES DISTRIBUTION OF YEAST ISOLATES

Introduction

Over the whole survey approximately 80% of the fish sampled, comprising sixteen species, have yielded yeast cultures. During the early stages of the survey yeasts were recovered from only 66% of the fish but in the later stages, with improved methods for sampling and isolation, recovery rose to between 98% and 100%. This seems indica-:tive that all fish caught in these northern waters can be expected to harbour yeasts in their micro-flora. For reasons mentioned earlier (see page 26) no attempt has been made in this survey to estimate quantitatively the yeast populations occurring on the fish but the indications are that relatively small numbers may be involved since no single site of sampling invariably gave positive results - see Table 30.

The various factors which may affect this yeast flora both quantitatively and qualitatively will now be discussed in view of the results obtained in this survey.

Geographical location of fishing ground

It can be seen from Table 27 that strains of D.kloeckeri were predominant among the isolates from every location of sampling, without exception. The occurrence of other species, however, and the proportion of isolates comprising them appear to be related mainly to the geographical location in which the fish were caught.

Such/

TABLE 27

Percentage of fish on which species were detected and percentage distribution of species in isolates as related to geographical areas:-

% Species occurrence in isolates				% Fish Positive for Species				
Atlantic off Iceland	North Sea	Clyde Estuary	Total Collection	Atlantic off Iceland	North Sea	Clyde Estuary	Total Collection	Species
35.0	50.5	46.0	49.0	92.0	75.0	40.0	56.0	Debaryomyces kloeckeri
3.0	9.0	-	4.0	8.0	15.5	-	5.5	Debaryomyces subglobosus
20.5	6.5	15.0	11.0	58.0	6.0	17.0	18.0	Candida parapsilosis
-	10.0	-	4.0	-	15.5	-	4.5	Candida lipolytica(var)
15.0	-	-	2.0	41.0	-	-	4.5	Candida species
-	6.5	26.0	11.0	-	9.0	20.0	15.0	Torulopsis inconspicua(var)
-	-	5.5	2.0	-	-	6.0	3.6	Torulopsis inconspicua
-	4.5	-	2.0	-	6.0	-	1.8	Torulopsis famata
-	2.5	1.5	2.0	-	3.0	1.5	1.8	Torulopsis candida
12.0	6.5	-	6.5	33.0	18.0	-	9.0	Rhodotorula glutinis var.rubescens
6.0	2.5	-	3.0	16.0	3.0	-	2.7	Rhodoturula mucilaginosa
3.0	-	-	0.5	8.0	-	-	0.9	Rhodotorula minuta
6.0	-	-	1.5	16.0	-	-	1.8	Rhodotorula rubra
-	-	3.0	1.0	-	-	3.0	1.8	Pichia membranaefaciens
-	2.5	3.0	2.0	-	6.0	3.0	3.6	Others

Such differences in the yeast flora of fish caught in the Clyde Estuary, in the Atlantic Ocean off the east coast of Iceland and in the North Sea are not altogether unexpected in view of the peculiar conditions existing in each region.

In the Clyde Estuary the micro-flora of the water surrounding the fish and hence of the fish itself must be greatly influenced by the close proximity of land and the fresh-water outflow. Both terrestrial and fresh-water yeasts will be introduced into the estuarine waters where they may survive for considerable periods and some may become so adapted that they are capable of propagating themselves in this new environment. Evidence that this may occur comes from the results of Capriotti (1962) [A,B] who recorded that eighty per cent., of the yeasts isolated by him from soils in coastal regions of Florida had also been found in estuarine waters and fifty per cent., in off-shore waters in the same region. The fact that Capriotti found a higher percentage of the terrestrial species in the estuarine waters is probably due to the more plentiful supply of organic material and favourable temperatures in these areas compared with those in off-shore waters. These factors may not only influence the survival of terrestrial yeasts but may promote an increase in the numbers of yeasts normally living in marine waters.

Notable features of the yeast flora of fish caught in the Clyde Estuary are the high incidence of Torulopsis spp., mainly T.inconspicua (var), and the absence of Rhodotorula spp., and Debaryomyces subglobosus, both/

both of which were isolated from fish caught in the other two areas. The absence of Rhodotorula spp., here is surprising in view of the reports in the literature of their widespread marine occurrence. However, the reliability of this observation is supported by the fact that no strains of Rhodotorula spp., were isolated from sea-water samples taken in the same area. Of note amongst the isolates from these sea-water samples were strains of Metschnikowia krissii (van Uden and Castelo-branco) nov.comb., and psychrophilic strains of Cryptococcus spp.

In the Atlantic, off the east coast of Iceland, probably the most important factor influencing the yeast flora is the North Atlantic Drift which originates from the Gulf Stream and brings supplies of easily assimilable organic material from the southern regions (Walford, 1958), and also raises the water temperature by a few degrees. In this connection it is of interest to note that in oceanic waters off South Florida, an area through which the Gulf Stream passes, Roth et al. (1962) found D.kloeckeri and C.parapsilosis to be the yeast species of the most widespread occurrence. In this survey the former species was found to have the greatest incidence of occurrence and to comprise the largest proportion of yeast isolates from fish caught in all three of the fishing grounds. C.parapsilosis, although also detected on fish from all these areas, was found in a significantly smaller proportion of fish in the North Sea, an area less influenced by/

by the Gulf Stream than the other two locations. However, until more information is available concerning the yeast flora of fish in areas not touched by the Gulf Stream the influence of this current can only be conjectured. Most notable in the yeast isolates from fish caught off Iceland is the high incidence of Rhodotorula spp., and the exclusive occurrence of strains resembling species of Candida.

Strains resembling Candida lipolytica and classified as C.lipolytica (var) were found only on the fish caught in the North Sea and, as mentioned above, the smaller incidence of C.parapsilosis than in the other areas is another feature of this region. Several of the species detected on fish in this area have also been detected in sea-weed and rock-pools found on the shore near Aberdeen (see Table 14) and also on salmon caught and sampled at Park, 14 miles up the River Dee, Aberdeenshire, i.e., Rh.glutinis var.rubescens, T.candida, Rh.mucilaginosa and D.kloeckeri. Since these fish were swimming up-river from the sea to spawn it may well be that the yeast flora carried by them was little changed from that when they were in the sea; if such is the case a more thorough investigation of their yeast flora in conjunction with that of the seas may help to elucidate some facts concerning the sea-life of this fish about which little is known.

The possible limitation of the occurrence of different strains of a particular species to certain geographical areas has also been studied using the groupings of each species in the Appendix as indications/

indications of possible strain variation within that species. D.kloeckeri is the only species whose isolates were able to be grouped in relatively large numbers and it can be seen that most of these groups contain isolates from at least two different areas, thus precluding the possible geographical limitation of similar strains of this species. However, further study of the yeast flora of sea-water in each region of sampling is necessary since, if it could be shown that no such overlapping of yeast grouping and geographic location occurs, then strain differences may be a guide to fish migration. Few of the isolates of the other species which occurred in different areas in large enough numbers to allow strain comparison were found to resemble each other, i.e. C.parapsilosis, Rh.glutinis var.rubescens and Rh.mucilaginosa, thus making strain comparison in the same and different areas for these species impossible. However, it is interesting to note that the few strains of T.incon-:spicua (var.) isolated in the North Sea fall into a single group which differed from the other groups comprising the isolates of this species from the Clyde Estuary.

Fish Species

As can be seen in Table 28, every type of fish sampled in this survey has yielded yeasts. A reasonable number of haddock, plaice, cod, sole, whiting and skate have been examined and the incidence of occurrence of yeasts from each kind of fish was never more or less than/

than ten per cent., from the average for the total number of fish examined, i.e., 80%. As smaller numbers only of dab, mackerel, witch, dogfish, saithe, hake, gurnard, bream, ling and turbot were examined there is no justification for an analysis of each type, but collectively yeasts were found on approximately 79% of these fish.

TABLE 28

Occurrence of yeasts on the different types of fish examined:-

Fish Species	Number Sampled: North Sea	Atlantic off Iceland	Clyde Estuary	Total	No.of fish with yeasts	Percentage of fish with yeasts
Haddock	4	3	13	22	16	73
Cod	2	3	12	17	13	76
Plaice	4	-	10	14	10	71
Sole	4	2	5	11	8	73
Whiting	3	-	7	10	9	90
Skate	4	-	3	9	7	78
N.Sea Dab	4	-	3	4	3	Collective percentage = 79
Ling	1	-	-	1	1	
Mackerel	4	-	4	4	4	
Witch	-	1	3	4	4	
Dogfish	-	-	3	3	2	
Gurnard	2	-	1	3	3	
Hake	1	-	2	3	2	
Saithe	-	1	2	3	2	
Bream	-	3	-	3	1	
Turbot	1	-	-	1	1	

The percentage occurrence of different yeast species amongst the isolates from a particular species of fish are presented in Table 29. All types of fish, except one of which only a single sample was available/

available, yielded cultures of D.kloeckeri in varying proportions of their total isolates, i.e., 25% - 100%.

Only cod and haddock, which were sampled in reasonably similar proportions in the different areas (see Table 28), can be compared with respect to their micro-flora at the same time eliminating the factor of geographical variation of the species. It is interesting to note, therefore, when such a comparison is made that a quantitative difference in the occurrence is indicated in the cases of C.parapsilosis and D.kloeckeri, i.e., 33% and 27%, respectively, on cod and 16% and 46% respectively, on haddock.

The fact that different fish species may carry their own charac-:teristic yeast flora still does not account for the geographical differences mentioned above as in some cases yeast strains found in relatively high numbers on a particular fish species in one area could not be detected on the same fish species in another area, e.g., Rhodotorula spp., found on the haddock, plaice, sole, cod, mackerel and hake (see Table 27) in the North Sea and Atlantic were not found on fish of the same species taken from the Clyde Estuary. Similarly, Torulopsis spp., were not found on fish taken from the Atlantic, off Icelend, although they were found on the same fish species in the North Sea and Clyde Estuary.

Using the groupings of the species in the Appendix to indicate possible/

TABLE 29

Species composition of isolates on each type of fish sampled

% of each yeast species of total isolates from each fish species										Total number isolates	Fish species
D.kloeckeri	D.subglobosus	C.parapsilosis	C.lipolytica (var.)	T.inconspicua (var.)	T.inconspicua	Candida Spp.	T.famata	T.candida	Rh.glutinis var.rubescens	Rh.mucilaginosa	Others		
46	10	16	7	10	3	-	-	-	7	-	-	30	Haddock
27	6	33	6	12	-	3	3	-	3	-	6	33	Cod
54	-	13	9	9	4	-	-	-	9	-	-	22	Plaice
46	8	12	-	-	8	4	4	-	12	-	4	24	Sole
33	-	9	-	45	-	-	-	14	-	-	9	22	Whiting
43	-	14	-	-	-	-	28	-	-	-	-	7	Skate
100	-	-	-	-	-	-	-	-	-	16	-	7	N.Sea Dab
58	8	-	-	8	-	-	-	-	8	-	-	12	Mackerel
50	-	12.5	-	25	-	-	-	-	-	-	12.5	8	Witch
100	-	-	-	-	-	-	-	-	-	-	-	3	Dogfish
60	-	-	20	20	-	-	-	-	-	-	-	5	Gurnard
50	-	-	33	-	-	-	-	-	16	-	-	6	Hake
43	-	14	-	-	-	14	-	-	-	25	14	7	Saithe
37	-	12.5	-	-	-	25	-	-	-	12.5	-	8	Bream
25	-	16	-	-	-	-	-	4	16	-	25	24	Salmon
-	-	-	-	-	-	-	-	-	-	-	100	1	Turbot
25	50	-	-	-	-	-	-	-	-	-	25	4	Ling

possible strain variation there appeared to be no link between type of strain and fish species from which they were isolated.

Location of sampling on fish

As was to be expected the incidence of yeast occurrence was found to be highest in the samples taken from the fish skin, followed by the gills and then in a considerably lower proportion, the faeces - see Table 30. As indicated in Table 30, mouth samples were taken only from fish caught in the North Sea and proved to have an almost similar yeast incidence as samples from the gills.

As can be seen in Table 31, the flora of the gills, skin and faeces appear to be substantially alike in specification although the total absence of T.inconspicua (var) in the skin isolates from fish caught in the North Sea is perhaps notable. Also of interest is the fact that the number of yeast species detected in the skin samples was greatest; another indication perhaps of the influence of sea-water on this location and also that of the trawling pro-:cedures. The slime layer covering the fish may also be conducive to the propagation of a varied yeast flora.

Conclusion

In view of the limited scope of this survey it is realised that findings described here regarding the influence of such factors as geographical location of fishing ground, species of fish and location of sampling on the fish, can only be taken as indications or clues

TABLE 30

No. and % samples from different locations on the fish yielding yeast cultures:-

Location of sampling on fish	Total Collection			Clyde Estuary			North Sea			Atlantic Ocean off Iceland		
	No. of samples	positive samples	% positive samples	No. of samples	positive samples	% positive samples	No. of samples	positive samples	% positive samples	No. of samples	positive samples	% positive samples
Mouth	22	15	68	-	-	-	22	15	68	-	-	-
Skin	69	60	87	34	21	62	28	22	79	7	7	100
Gills	96	54	55	65	31	48	27	19	70	4	4	100
Faeces	96	36	37	65	20	31	27	13	48	4	3	75

N.B. Blank spaces indicate that no samples were taken at those locations.

TABLE 31

Percentage occurrence of different species of yeast in different sampling locations on the fish arranged according to areas of catching fish:-

yeast species	% occurrence in gill isolates					% occurrence in skin isolates					% occurrence in faeces isolates					% occurrence in mouth isolates				
	Total	Clyde Estuary	North Sea	Atlantic, off Iceland	Miscellaneous	Total	Clyde Estuary	North Sea	Atlantic, off Iceland	Miscellaneous	Total	Clyde Estuary	North Sea	Atlantic, off Iceland	Miscellaneous	Total	Clyde Estuary	North Sea	Atlantic, off Iceland	Miscellaneous
D.kloeckeri	49	45	70	26.5	45	43	50	50	35	15.5	41	43	46	50	-	55.5	not sampled	58	not sampled	not sampled
D.subglobosus	-	-	-	-	-	6	-	10	6	8	2	-	8	-	-	17		18		
C.parapsilosis	10	13	-	26	-	11	15	7	23	-	18	19	-	-	100	-		-		
C.lipolytica(var)	3	-	10	-	-	5	-	14	-	-	4.5	-	15	-	-	5.5		6		
Candida spp.	3	-	-	18	-	4	-	-	18	-	-	-	-	-	-	-		-		
T.inconspicua(var)	20	39	10	-	-	4	15	-	-	-	13.5	19	15	-	-	5.5		6		
T.inconspicua	1	3	-	-	-	1	5	-	-	-	4.5	9	-	-	-	-		-		
T.famata	3	-	-	-	22	1	-	3	-	-	-	-	-	-	-	5.5		6		
T.candida	3	-	5	-	11	-	-	-	-	-	4.5	5	8	-	-	-		-		
Rh.glutinis var. rubescens	3	-	-	9	11	10	-	14	6	23	7	-	8	33	-	5.5		6		
Rh.mucilaginosa	4	-	5	18	-	6	-	3	-	30	-	-	-	-	-	-		-		
Rh.minuta	-	-	-	-	-	1	-	-	6	-	-	-	-	-	-	-		-		
Rh.rubra	-	-	-	-	-	1	-	-	6	-	2	-	-	17	-	-		-		
P.membranaefaciens	-	-	-	-	-	1	5	-	-	-	2	5	-	-	-	-		-		
Trichosporon spp.	-	-	-	-	-	1	-	-	-	8	-	-	-	-	-	-		-		
P.pullulans	-	-	-	-	-	2.5	-	-	-	15.5	-	-	-	-	-	-		-		
Others	1	-	-	-	11	2.5	10	-	-	-	-	-	-	-	-	5.5		5.5		

to the true picture of the qualitative and quantitative occurrence of yeasts on marine fish. From bacteriological studies of marine fish there is, however, some evidence to support the indications of the results obtained in this study. The fact that more mesophilic bacteria have been found on fish caught in warmer waters than in those waters around Britain (Shewan, 1961) where more psychrophilic types have been found, does indicate that a geographical distribution can occur, at least based on temperature difference.

The occurrence of characteristic bacterial florae on fish is, however, a more controversial point. Wood (1953) stated that the micro-florae of Australian teleosts were characterised by a large number of Micrococci and that of the elasmobranchs by a preponderance of Coryneforms and he beleived that this difference was related to the constitution of the slime layers which varied from fish to fish. Liston (1957, 1955), on the other hand, found no such marked differences between the micro-florae of skate and sole, an elasmo-branch and teleost respectively, which were caught off Aberdeen. Working with fresh-water fish Potter and Baker (1961) also indicated that they found no relationship between the composition of micro-flora isolated and the type of fish sampled. Recently, however, Liston and Colwell (1961) reported that they had found evidence to indicate that the bacterial flora of marine invertebrates is related to the species of animal.

Another/

Another factor influencing the micro-flora of fish is that of season. Bacteriological evidence suggests that not only is the micro-flora of fish affected quantitatively by this factor (Shewan, 1961) but also qualitatively (Liston, 1955: Georgala, 1958). De Silva (1960) reported that higher numbers of yeasts were detected in the North Sea in July, 1958 than in the following October and if such variations also occur qualitatively this could explain in part the difference in yeast flora obtained in the various grounds during this survey, i.e., collections were made in the Clyde Estuary in May and June, in the North Sea in July and in the Atlantic in September.

It seems, therefore, in view of all the variables discussed, that only by increasing the scope of investigation along the lines of the present study can the role of these factors be elucidated.

S U M M A R Y

In the Introduction, literature regarding the occurrence and composition of the yeast flora of marine fish and other parts of the marine environment has been reviewed.

The experimental work is divided into three sections:-

1) Collection and maintenance of yeasts from the marine environment

2) Identification of isolates according to the system of Lodder and Kreger-van Rij(1952)

3)Further studies of the properties of these isolates

1) Altogether from various marine sources 235 yeast cultures have been collected and of these, during this survey, 189 were isolated from marine fish and 11 from sea-water. The remaining 35 were contributed to the collection by other workers. Improved methods for sampling and isolation of these yeasts are described.

2) The 213 isolates from marine fish were found to comprise eight genera - Debaryomyces, Torulopsis, Candida, Rhodotorula, Pichia, Trichosporon, Cryptococcus and Pullularia. Forty-five percent. of these isolates were classified as D.kloeckeri. T.inconspicua(var) and C.parapsilosis each comprised 11% respectively.

The 22 isolates from other marine sources belonged to the same genera as the fish isolates and in addition five of the eleven strains isolated from sea-water samples in this survey were classified as Metschnikowia krissii(vanUden and Castelo-branco)nov.comb.

Although most of the isolates agreed closely with the descriptions of the type species some differences were obvious and these have been discussed in the light of possible strain variation within the species.

3) All the strains were subjected to further tests other than those proposed by Lodder and Kreger-van Rij (1952). The results of these tests and their probable value in establishing strain variation are discussed.

The use of infra-red spectrophotometry in the study of the yeast isolates has been investigated and the results indicate that the spectra of whole cells, soluble and insoluble cellular material are of little value for species or strain differentiation. It was conjec-:tured that identical cellular components producing specific spectra occur in the different species and mask the effects of differences in other cellular components.

The free-amino-acid pools of certain of the marine isolates and terrestrial strains of corresponding species were found to contain at least 14 amino-acids. Apart from differences in the occurrence of acids present only in trace quantities, no qualitative differences in the composition of the pools of the marine and terrestrial strains were found and thus strain characterisation on this basis was impracti-:cable.

The maximum concentration of NaCl tolerated by the marine isolates was found to be a feature of each strain and there was a correlation regarding halo-tolerance among strains of a particular species isolated from/

from different marine locations. Complex organic nitrogen sources were found to stimulate halo-tolerance in the isolates. As the concentration of NaCl was increased in the growth medium a prolonga-:tion in the yeasts' lag phase was observed but their growth rate remained fairly constant. Strains of D.kloeckeri were found to exhibit optimal growth in media containing 1.0 to 3.0% NaCl, whereas the other species tested grew best without NaCl.

The thesis concludes with a discussion concerning the occurrence and species distribution of the yeasts isolated during this survey.

Skin samples had the highest incidence of yeast occurrence; the samples from the gills and mouth exhibited the next highest incidence of yeast and were followed by those from the faeces where a much lower value was recorded.

Without exception D.kloeckeri was the predominant species in every geographical location of sampling, whereas the occurrence of the other species and the proportion of isolates comprising them varied within these areas. Results also indicate that different fish species may carry their own characteristic yeast flora.

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REFERENCES

Adams, A.M., (1950) Can.J.Res., Sect.F, 413

Ahearn, D., and Roth, F.J. (1962) in "Developments in Industrial Microbiology", Vol.3, Plenum Press Inc., 227 West 17 Street, New York

Atkin, L.A., Moses, W., and Gray, P.P. (1949) J.Bact., 57, 575

Baldwin, E., (1949) in "Introduction to Comparative Biochemistry", Camb.Univ.Press

Barnett, J.A., (1960) Nature, 188, 4723

Barnett, J.A., and Ingram, M., (1955) J.Appl.Bact., 18, 131

Beech, F.W., and Carr, J.G., (1955) J.Gen.Microbiol., 12, 85

Beech, F.W., Carr, J.G., and Codner, R.C., (1955) J.Gen.Microbiol., 13, 408

Benedict, A.A., Pollard, M., and Engley, F.B., (1954) Texas Reports on Biology and Medicine, 12, 21

Berridge, N.J., Cheeseman, G.C., and Mattick, A.T.R., (1957) J.Appl. Bact., 20, 195

Bhat, J.V., and Kachwalla, N., (1955) Proc. Indian Acad.Sci., 41(B), 9

Bhat, J.V., Kachwalla, N., and Mody, B.N., (1955) J.Sci. Ind.Res., India, 14C, 24

Bissett, K.A., (1948) J. Hyg., 46, 94

Brady, B.L., (1959) Rec.Res. in Freezing and Drying, 243.

Brealey, L., and Elvidge, D.A., (1956) J.Pharm.Pharmacol., 8, 885

Brown, M.E., (1951) in "Physiology of Fishes", Vol.I, Academic Press Inc., New York

Bulder, C.J.E.A., (1955) Antonie van Leeuwenhoek, J.Microbiol. Serol., 21, 434

Capriotti, A., (1962)[A] Arch. Mikrobiol., 41, 142

Capriotti, A., (1962)[B] Arch. Mikrobiol., 41, 147

(1962)[C] Arch. Mikrobiol., 42, 407

Castell, C.H., and Mapplebeck, E.G., (1952) J.Fish Res.Bd., Can., 9, 377

Cawley, E.P., et al., (1954) J.Invest.Dermatol., 22, 273

Cheeseman, G.C., (1959) J.Appl.Bact., 22, 341

Conway, E.J., and Moore, P.T., (1952) in 2nd Congr.Inst.Biochemie, Resumés des Communications, page 14

Costilow, R.N., Etchalls, J.L., and Blumer, T.N., (1954) Appl. Microbiol., 2, 300

Cutting, C.L., (1955) in "Fish Saving", Leonard Hill, Ltd., London, N.W.1.

de Silva, N.N., (1960) in "A Study of the Bacteriology of the North Sea Herring and the Effects of Certain Antibiotics such as Chlortetracycline and Oxytetracycline on the Flora and Spoilage Characteristics of Such Herring", Ph.D. Thesis, Univ.Aberdeen.

di Menna, M.E., (1960) J.Gen.Microbiol., 23, 295

di Menna, M.E., (1962) J.Gen.Microbiol, 27, 249

Diddens, H.A., and Lodder, J., (1942) "Die anaskosporogenen Hefen, II Hälfte", North Holland Publ., Amsterdam

Drake, S.O., Evans, J.B., and Niven, C.F., (1959) Food Res., 24, 243

Dunn, C.G., (1952) Wallerstein Labs.Communs., 15, 61

Dussault, H.P., and Lachance, R.A., (1952) J.Fish Res.Bd., Can., 9, 157

Dyer, F.E., (1947) J.Fish Res.Bd., Can., 7, 128

Etchells, J.L., and Bell, T.A., (1950) Farlowia, 4, 87

Etchells, J.L., Costilow, R.N., and Bell, T.A., (1952) Farlowia, 4, 249

Etchells, J.L., Bell, T.A., and Jones, I.D., (1953) Farlowia, 4, 265

Etchells, J.L., Costilow, R.N., Bell, T.A., and Delmain, A.L. (1954) Appl.Microbiol., 2, 296

Etchells, J.L., Borg, A.F., and Bell, T.A., (1961) Appl.Microbiol. 9, 139

Fell, J.W., Ahearn, D.G., Meyers, S.P., and Roth, F.J., (1960) Limnol. and Oceanogr., 5, 366

Fell, J.W., and Roth, F.J., (1961) Bact.Proc., 59

Fell, J.W., and van Uden, N., (1961) Bact.Proc., 40

Fischer, B., (1894) Zeitschr.f.Hyg., 17, 185
cit.: Zobell (1946)

Fischer, B., and Brebeck, C., (1894) in "Zur Morphologie, Biologie und Systematik der Kahmpilze, der Monilia candida Hansen und des Soorerregers" (G.Fischer, Jena)
cit.: Zobell (1946)

Forster, J., (1887) Centralbl.f.Bakt., 2, 337
cit.: Zobell (1946)

Fry, R.M., and Greaves, R.I.N., (1951) J.Hyg. (London), 49, 220

Gale, E.F., (1947) J.Gen.Microbiol., 1, 53

Georgala, D.L., (1957) in "Quantitative and Qualitative Aspects of the Skin Flora of North Sea Cod and the Effect Thereon of Handling on Ship and on Shore", Ph.D. Thesis, Univ.Aberdeen

Georgala, D.L., (1958) J.Gen.Microbiol., 18, 84

Gibbons, N.E., (1934) Contr.Canad.Biol.Fish, 8, 291

Goulden, J.D.S., and Sharpe, M.E., (1958) J.Gen.Microbiol., 19, 76

Graham, V.E., and Hastings, E.G., (1942), Can.J.Res., 20, 63

Greenstreet, J.E.S., and Norris, K.P., (1957) Spectrochim.Acta, 9, 177

Halvorson, H.O., and Spiegelman, S., (1953) J.Bact., 65, 601

Halvorson, H.O., Frey, W., and Schwemmin, D., (1954-55), J.Gen. Physiol., 38, 549

Hardy, A., (1959) in "The Open Sea. Its Natural History, Part II, Fish and Fisheries", Collins, St. James Place, London

Harris, G., (1958) in "The Chemistry and Biology of Yeasts, Ch.IX, ed. A.H. Cook, Academic Press Inc., New York

Hartelius, V., and Ditlevson, E., (1953) Compt.rend.trav.lab. Carlsberg Sér., physiol., 25, 213

Hasegawa, T., Banno, I., and Yamauchi, S., (1960) J.Gen.Appl. Microbiol., 6, 196

Haynes, W.C., Wickerham, L.J., and Hesseltine, C.W., (1955), Appl.Microbiol., 3, 361

Haynes, W.C., Melvin, E.H., Locke, J.M., Glass, C.A., and Senti, F.R. (1958), Appl.Microbiol., 6, 298

Hertz, M.R., and Levine, M., (1942) Food Res., 7, 430

Hine, R.B., (1960) Mycologia, 52, 378

Hof, T., (1935) Rec.Trave.Bot.Neerland, 32, 92

Hunter, A.C., (1920) U.S.Dept.Agric.Bull., No. 819, 1

Ingram, M., (1938) Rept.Fd.Invest.Board, London, 72

Ingram, M., (1957) in 7th Symposium Soc.Gen.Microbiol., Camb, Univ.Press

Issachenko, B.L., (1914) Izbrannye Trudy Inst., Akad,Nauk., SSSR, Moscow, 111
cit.: Johnson and Sparrow (1961)

Issachenko, B.L., (1929) Mikrobiologicheskiyi Zhurnal, 9, 78
cit.: Johnson and Sparrow (1961)

Johnson, T.W., and Sparrow, F.K. (1961) in "Fungi in Oceans and Estuaries", J.Cramer, publisher in Weinheim

Joslyn, M.A., (1927) Fruit Prods.,L.and Amer.Vin.Industr., 6, 22

Joslyn, M.A., and Cruess, W.V., (1929-30) Hilgardia, 4, 201

Katznelson, H., and Lochhead, A.G., (1952) J.Bact., 64, 97

Kenner, B.A., Riddle, J.W., Rockwood, S.W., and Bordner, R., (1958) J.Bact., 75, 17

Kirsop, B.H., (1955) J.Inst.Brew., 61, 466

Klaushofer, H., et al., (1961) Mitt.Versuchs, Garungsguv., Wien, 15, 10
cit.: J.Inst.Brew.,(1961), 67, 294

Kobayasi, Y., Tsubaki, K., and Soneda, M., (1953) Bull.Nat.Sci. Museum, Tokyo, No.33, 47
cit.: Johnson and Sparrow (1961)

Konokotina, A.G., and Krassihukow, V.A., (1929) J.Microbiol. Leningrade, 9, 93

Krasil'nikova, E.N., (1962) Mikrobiologiya, 30, 651

Kreger, D.R., (1954) Biochem. et Biophys. Acta, 13, 1

Kreger-van Rij, N.J.W., (1962) Symposia of the Socy.Gen.Microbiol., No. XII, 196

Kriss, A.E., Rukina, E.A., and Tikhonenko, A.S., (1952) Zhur. Obschii. Biol., Akad.Nauk., SSSR, 13, 232

Kriss, A.E., and Novozhilova, M.I., (1954) Mikrobiologiya, 23, 669

Kriss, A.E., Biriuzova, V.I., Tikhonenko, A.S., and Lameuna, V.A., (1955) Doklad.Akad.Nauk., SSSR, 101, 173

Kriss, A.E., (1959) in "Marine Microbiology", Akad.Nauk., USSR, Moscow
English translation publ. Oliver and Boyd, Edinburgh (1962)

Kubitschek, H.E., (1958) Nature, 182, 234

Kudriavtzev, V.I., (1932) Doklad. Akad. Nauk. SSSR, 12, 292
cit.: Rukina and Novozhilova (1952)

Kudriavtzev, V.I., (1933) Vestnik. Dal'nevost filiala Akad.Nauk. SSSR, No. 1-3, 99
cit.: Rukina and Novozhilova (1952)

Kudriavtzev, V.I., (1954) in "Yeast Systematics" (in Russian), Akad. Nauk. USSR, Moscow

Larson, H., (1954) in "Autotrophic Micro-organisms", 4th Sympos. Socy.Gen.Microbiol., Camb.Univ.Press

Lawrence, N.L., Wilson, D.C., and Pederson, C.S., (1959) Appl. Microbiol., 7, 7

Lederberg, J., and Lederberg, E.M., (1952) J.Bact., 63, 399

Levine, S., Stevenson, H.C.R., and Bordner, R.H., (1953) Science 118, 141

Levine, S., Stevenson, H.J.R., Chambers, L.A., and Kenner, B.A., (1953), J.Bact., 65, 10

Lindan, O., and Work, E., (1951) J.Biochem., 48, 337

Liston, J., (1955) in "A quantitative and qualitative study of the bacterial flora of skate and lemon sole trawled in the North Sea", Ph.D. Thesis, Aberdeen University

Liston, J., (1956) J.Gen. Microbiol., 15, 305

Liston, J., (1957) J.Gen. Microbiol., 16, 205

Liston, J., and Colwell, R.R., (1961) Bact.Proc., D14, 47

Lodder, J., (1934) "Die anaskosporogen Hefen, I Hälfte", Verhandel.Koninkl.Akad.Wetenschap.Afdel.Natuurk. Sect.II, 32, 1
cit.: Lodder et al., (1958)

Lodder, J., and Kreger-van Rij, N.J.W., (1952) in "The Yeasts", North Holland Publ., Amsterdam

Lodder, J., Slooff, W.Ch., and Kreger-van Rij, N.J.W., (1958) in "The Chemistry and Biology of Yeasts", Ch.I, ed. A.H.Cook, Academic Press Inc., New York

Lund, A., (1954) in "Studies on the Ecology of Yeasts", Munksgaard, Copenhagen

Mandelstam, J., (1958) J.Biochem., 69, 103

Margolis, L., (1953) J.Fish Res.Bd.,Can., 10, 62

Mattick, A.T.R., Cheeseman, G.C., and Berridge, N.J., (1956) J.Appl.Bact., 19, 310

McCormack, G., (1950) Fish and Wildlife Service, U.S.Dept.of Interior Bull., 12, No. 11A

McCormack, G., (1956) Comm.Fish Rev., 18, No.11, 21

McLachlan, J., (1961) Can.J.Microbiol., 7, 399

McLeod, R.A., and Onofrey, E., (1956) J.Bact., 71, 611

Meiklejohn, J., (1950) J.Gen.Microbiol., 4, 188

Merdinger, E., and Shair, S., (1962) Can.J.Microbiol., 8, 213

Metschnikoff, E., (1884) Vischows.Arch., 96, 1777
Cit.: van-Uden and Castelo-branco (1961)

Miles, A.A., and Misra, S.S. (1938) J.Hygiene, 38, 732

Miller, M.W., and Phaff, H.J., (1958) Antonie van Leeuwenhoëk J.Microbiol.Serol., 24, 225

Morris, E.O., (1958) in "The Chemistry and Biology of Yeasts", Ch. VI, ed. A.H. Cook, Academic Press Inc., New York

Mrak, E.M., and Bonar, L., (1938) Food Res., 3, 615

Mrak, E.M., and Bonar, L., (1939) Zentr.Bakt.Parasitenk, 100, 289

Mrak, E.M., and Phaff, H.J., (1948) Ann.Rev.Microbiol., 2, 1

Nadson, G., and Burgvitz, G., (1931) Compt.Rend.Acad.Sci. USSR, 103

Nagai, S., (1953) J.Inst.Polytech.Osaka City Univ., 4, Ser.D, 35

Nakazima, M., (1954) Kaguku (Science) 24, 61

(1955) Bull.Biogeogr.Soc. Japan, 16

(1957) Japanese J.Bot., 32, 261
Cit.: Johnson and Sparrow (1961)

Norris, K.P., (1953) VIth Int.Congr.of Microbiology, Rome, Vol.I, V, 807

Norris, K.P., and Greenstreet, J.E.S., (1958) J.Gen.Microbiol., 19, 566

Novak, E.K., and Zsolt, J., (1961) Acta Botanica Acad.Scient. Hungaricae, 7, 93

Novozhilova, M.I., (1955) Trudy Inst.Mikrobiol.,Akad.Nauk. SSSR, 4, 155

Onishi, H., (1959) Rep.Noda.Inst.Sci.Res., Japan, 49

Phaff, H.J., Mrak, E.M., and Williams, O.B., (1952) Mycologia, 44, 431

Phaff, H.J., (1956) Antonie van Leeuwenhoëk J.Microbiol.Serol., 22, 113

Potter,L.F., and Baker, G.E., (1961) Can.J.Microbiol., 7, 595

Pridham, T.G., (1952) Econ.Bot., 6, 185

Provasoli, L., (1958) Ann.Rev.Microbiol., 12, 279

Reay, G.A., and Shewan, J.M., (1949) Adv.Fd.Res., 2, 343

Redfield, R.R., (1953) Biochem.Biophys.Acta, 10, 344

Reed, G.B., and Spence, C.M., (1929) Contribs.Can.Biol.and Fisheries, 4, 259

Reiersöl, S., (1958) Antonie van Leeuwenhoëk J.Microbiol.Serol., 24, 111

Riddle, J.W., Kabler, P.W., Kenner, B.A., Bordner, R.H., Rockwood, S.W., and Stevenson, H.J.R., (1956) J.Bact., 72, 593

Ritchie, D., (1959) Bull.Torrey.Bot.Club, 86, 367

Roberts, C., and Thorne, R.S.W., (1960) Nature, 188, 873

Roth, F.J., Ahearn, D.G., Fell, J.W., Meyers, S.P., and Meyer, S.A., (1962) Limnol.Oceanogr., 7, 178

Rukina, E.A., and Novozhilova, M.I., (1952) Trudy Inst. Microbiol., No. 2, 150

Sanborn, J.R., (1930) J.Bact., 19, 375

Santa-maria, J., (1960) Nature, 188, 4715

Schultz, A.S., and Atkin, L., (1947) Arch.Biochem., 14, 369

Scopes, A.W., (1962) J.Gen.Microbiol., 28, 69

Scott, W.J., (1937) J.Council Sci.Ind.Res., 10, 338

Shewan, J.M., (1949) J.Roy.San.Inst., 59, 394

Shewan, J.M., (1961) in "Fish as Food", ch.14, 487 ed. G.Borgstrom, Academic Press Inc., New York

Shifrine, M.H.J., Phaff, H.J., and Demain, A.L., (1954) J.Bact., 68, 28

Siepmann, R., (1959) Veröff.Inst.Meeresforsch Bremerhaven, 6, 283

Siepmann, R., and Höhnk, W., (1962) Veröff.Inst.Meeresforsch, Bremerhaven, 8, 79

Simon, S., and Hedrick, L.R., (1955) J.Bact., 69, 4

Skinner, C.E., and Huxley, M.J., (1956) Mycologia, 371

Snow, J.E., and Beard, P.J., (1939) Food Res., 4, 563

Spencer, R., (1961) J.Appl.Bact., 24, 4

Spiegelman, S., et al., (1955) in "Amino acid metabolism", McElroy, W., and Glass, B., John Hopkins, Baltimore

Stelling-Dekker, N.M., (1931) Verhandel, Koninkl.Akad.Wetenschap. Afdel.Natuurk, Sect.,II, 28, 1
Cit.: Lodder et al., (1958)

Stevenson, H.J.R., and Balduan, O.E.A., (1952) Science, 116, 111

Stewart, M.M., (1932) J.Mar.Biol.Assoc., 18, 35

Straka, R.P., and Stokes, J.L., (1960) J.Bact., 80, 622

Swenson, P.A., and Dott, D.H., (1961) J.Cell.Comp.Physiol., 58, 217

Subramaniam, M.K., and Prahlada Rao,L.S., (1951) Experienta 7, 98

Tabey-Shehata, A.M., (1960) Appl.Microbiol., 8, 73

Takada, H., (1956) J.Inst.Polytech.,Osaka City Univ., 7D, 95

Takada, H., and Tokuno, S., (1958) J.Inst.Polytech., Osaka City Univ., 9D, 27

Tarr, H.L.A., (1954) Bact.Revs., 18, 1

Taylor, E.S., (1947) J.Gen.Microbiol., 1, 86

Taylor, E.S., (1949) J.Gen.Microbiol., 3, 211

Thomas, L.C., and Greenstreet, J.E.S., (1954) Spectrochim.Acta, 6, 302

Tolberg, A.B., and Pace, N., (1960) Proc.Soc.Exp.Biol.Med.,103, 488

Usden, U.R., and Burrell, R.C., (1952) Arch.Biochem.Biophys., 36, 172

Vaisey, E.B., (1954) J.Fish Res.Bd., Canada, 11, 90

Van der Walt, J.P., and van Kerken, A.E., (1960) Antonie Leeuwenhoëk, J.Microbiol.Ser., 26, 201

Van der Walt., J.P., and van Kerken, A.E., (1961) Antonie Leeuwenhoëk, J.Microbiol.Ser., 27, 206

van-Uden, N., and Castelo-branco, R., (1961) J.Gen.Microbiol., 26, 141

van-Uden, N., and Kolipinski, M.C., (1962) Antonie van Leeuwenhoek, J.Microbiol.Ser.,28, 78

Vishniac, H.S., (1960) Limnol.Oceanogr., 5, 362

Walford, L.A., (1958) in "Living Resources of the Sea", The Ronald Press Co., New York

Walker, H.W., and Ayres, J.C., (1959) Appl.Microbiol., 7, 251

Wickerham, L.J., and Andreason, A.A., (1942) Wall.Lab.Communs., 5, 165

Wickerham, L.J., Fleckinger, M.H., and Burton, K.A., (1946) J. Bact., 52, 611

Wickerham, L.J., (1951) in"Taxonomy of Yeasts", U.S.Dept.Agric. Tech.Bull.,No.1029

Wickerham, L.J., and Burton, K.A., (1952) J.Bact., 63, 449

Willis, A.T., (1960) J.Path.Bact., 80, 379

Wood, E.J.F., (1940) Australia Commonwealth Council Sci.Ind.Res. Div.Fisheries Dept., No. 3

Wood, E.J.F., (1950) Austr.J.Mar.Fresh Water Res., 1, 129

Wood, E.J.F., (1953) " " " " , 4, 111

Wynne, E.S., and Gott, C.L., (1956) J.Gen.Microb., 14, 512

Zenitani, B., (1952) Kyushu Univ., Sci.,Bull.Fac.Agric., 12, 383

Zobell, C.E., and Feltham, C.B., (1934) Bull.Scripps Inst.Oceanogr. Tech.Ser., 3, 279

Zobell, C.E., (1941) J.Mar.Res., 4, 173

Zobell, C.E., and Upham, H.C., (1944) Bull.Scripps.Inst.Oceanogr. Univ.Calif., 5, 239

Zobell, C.E., (1946) in "Marine Microbiology", Chronica Botanica Co., Waltham, Mass., USA

Zobell, C.E., (1959) in "Contributions to Marine Microbiology", compiled T.Skerman, New Zealand DSIR, Information Series No. 22

Zsolt, J., (1958) Antonie van Leeuwenhoek, J.Microbiol.Serol., 24, 210

Zsolt, J., (1959) Acad.Scient.Hungaricae, 233

A P P E N D I X

CLASSIFICATION OF ISOLATES

Strain No.	Classification
2	Candida parapsilosis
7	Debaryomyces kloeckeri
8	Candida parapsilosis
11	Debaryomyces kloeckeri
13	Candida parapsilosis
15	Debaryomyces kloeckeri
21	Candida parapsilosis
24	Debaryomyces kloeckeri
31	Candida parapsilosis
34	Debaryomyces kloeckeri
39	" "
41	" "
42	Torulopsis candida
43	Debaryomyces kloeckeri
46	" "
49	" "
50	" "
52	" "
53	" "
59	" "
62	Candida parapsilosis
63	Debaryomyces kloeckeri
65	Torulopsis inconspicua
66	" "
67	Torulopsis famata
68	Debaryomyces kloeckeri
102	Candida parapsilosis
103	Debaryomyces kloeckeri
104	Torulopsis inconspicua
107	Torulopsis inconspicua(var)
108	" " "
112	" " "
115	" " "
117	Candida parapsilosis
120	" "
130	Debaryomyces kloeckeri
131	" "
132	" "
134	Torulopsis inconspicua(var)
136	" "
146	Debaryomyces kloeckeri
147	" "
148	" "
150	Torulopsis inconspicua
157	Debaryomyces kloeckeri
158	Candida parapsilosis
159	Debaryomyces kloeckeri
160	Torulopsis inconspicua(var)
161	Debaryomyces kloeckeri
164	" "
165	" "
166	Torulopsis inconspicua(var)
167	" " "
169	Debaryomyces kloeckeri
170	Torulopsis inconspicua(var)
175	" " "
176	Debaryomyces kloeckeri
177	" "
179	Unidentified
181	Torulopsis inconspicua(var)
182	Unidentified
183	Debaryomyces kloeckeri
185	" "
187	Torulopsis inconspicua(var)
188	Pichia membranaefaciens
189	" "
190	Torulopsis inconspicua(var)
191	Candida parapsilosis
192	Torulopsis inconspicua(var)
193	" " "
194	" " "
195	Debaryomyces kloeckeri
196	Torulopsis inconspicua(var)
197	" " "
301	Debaryomyces kloeckeri
302	" "
302p	Rhodotorula glutinis var. rubescens
303	Debaryomyces subglobosus
304	" "
305	Debaryomyces kloeckeri
306	" "
307	" "
308s	Torulopsis famata
308m	Debaryomyces kloeckeri
309w	Candida parapsilosis

Strain No.	Classification
309p	Rhodotorula glutinis var. rubescens
311	Debaryomyces kloeckeri
314	Candida parapsilosis
316	Debaryomyces kloeckeri
317w	" "
317p	Rhodotorula glutinis var. rubescens
318	Torulopsis inconspicua(var)
319	Debaryomyces kloeckeri
320	" "
321w	" "
321p	Rhodotorula glutinis var. rubescens
322	Debaryomyces kloeckeri
325	Debaryomyces subglobosus
327	" "
329w	Debaryomyces kloeckeri
329p	Rhodotorula mucilaginosa
330w	Torulopsis candida
330p	Rhodotorula mucilaginosa
331	Debaryomyces kloeckeri
332p	Rhodotorula glutinis var. rubescens
333	Debaryomyces kloeckeri
335	" "
336	" "
337	Debaryomyces subglobosus
338	Debaryomyces kloeckeri
340	Torulopsis candida
341s	Debaryomyces kloeckeri
341m	" "
342	" "
343	" "
344	" "
345	" "
347	" "
348	" "
354L	Candida lipolytica(var)
354s	Debaryomyces kloeckeri
360	" "
370	Candida lipolytica(var)
371	Debaryomyces kloeckeri
202	Debaryomyces subglobosus
204	" "

Strain No.	Classification
207	Debaryomyces kloeckeri
208	" "
209	" "
212	" "
213	Torulopsis inconspicua(var)
214	Debaryomyces kloeckeri
215	Torulopsis inconspicua(var)
219	Debaryomyces kloeckeri
222	" "
224	" "
226	Candida lipolytica(var)
227	Debaryomyces kloeckeri
228	Candida lipolytica(var)
230	Candida " "
230w	Torulopsis famata
231	Debaryomyces kloeckeri
233	Candida lipolytica(var)
235	Debaryomyces kloeckeri
236	" "
238	Candida lipolytica(var)
240	Torulopsis inconspicua(var)
242	Debaryomyces kloeckeri
245	Torulopsis inconspicua(var)
246s	Debaryomyces kloeckeri
246L	Candida lipolytica(var)
248p	Rhodotorula glutinis var. rubescens
248w	Debaryomyces kloeckeri
249	Candida zeylanoides
417m	Debaryomyces kloeckeri
417s	Candida species
417p	Rhodotorula minuta
418	Debaryomyces kloeckeri
419m	" "
419s	Candida parapsilosis
419H	Candida species
420m	Debaryomyces kloeckeri
420s	Candida parapsilosis
420H	Candida species
421s	Debaryomyces kloeckeri
421m	Candida species
421H	Candida parapsilosis
422m	Debaryomyces subglobosus
422s	Candida parapsilosis
422p	Rhodotorula glutinis var. rubescens

Strain No.	Classification
423m	Debaryomyces kloeckeri
423s	Candida parapsilosis
423p	Rhodotorula glutinis var. rubescens
424s	Debaryomyces kloeckeri
424H	Candida species
424p	Rhodotorula rubra
425	Debaryomyces kloeckeri
427m	" "
427s	Candida species
427r	Rhodotorula mucilaginosa
427p	" "
428w	Debaryomyces kloeckeri
428p	Rhodotorula glutinis var. rubescens
429	Candida parapsilosis
443w	Debaryomyces kloeckeri
443p	Rhodotorula glutinis var. rubescens
450p	Rhodotorula rubra
450w	Debaryomyces kloeckeri
402	Torulopsis pseudaria
405	Debaryomyces subglobosus
407	Cryptococcus diffluens
Y3	Debaryomyces kloeckeri
Y11	" "
Y54	" "
254	Rhodotorula glutinis var. rubescens
279	Torulopsis candida
281	Debaryomyces kloeckeri
431	" "
1108	Rhodotorula rubra
1109	Rhodotorula mucilaginosa
1110	" "
1111	" "
1112	Rhodotorula glutinis var. rubescens
1113	Trichosporon pullulans
1114	Debaryomyces kloeckeri
1131	Rhodotorula glutinis var. rubescens
1301	Rhodotorula glutinis var. rubescens
BY1	Pullularia pullulans
BY2	" "
T2	Candida parapsilosis
T4	" "
T6	" "
T7	" "
846	Torulopsis famata
845	" "
40red	Rhodotorula glutinis var. rubescens
T40	Torulopsis candida
12pink	Rhodotorula glutinis var. ruboscens
LightI	Candida parapsilosis
LightII	" "
43/6	Torulopsis pseudaria
117A	Pullularia pullulans
Her3	Candida lipolytica(var)
Her7	" " "
501	Metschnikowia krissii
502	Trichosporon cutaneum var. multisporum
503	Trichosporon krissii var. multisporum
504	Debaryomyces kloeckeri
505	Metschnikowia krissii
506	" "
507	Cryptococcus diffluens
508	Metschnikowia krissii
509	" "
510	Cryptococcus albidus
511	Cryptococcus laurentii

COMPARISONS OF THE VARIOUS ISOLATES COMPRISING THE YEAST COLLECTION WITH DESCRIPTION OF THE TYPE SPECIES AND GROUPINGS OF THE ISOLATES WITHIN THESE SPECIES ACCORDING TO THEIR DIFFERING PROPERTIES:-

A) Debaryomyces kloeckeri

Type species - after Lodder and Kreger-van Rij(1952)

The ninety-eight isolates fitting the description of the type species all required biotin for growth, were unable to split fat nor liquefy gelatin and utilised the following additional carbon compounds - xylose, raffinose, L-arabinose, D-mannitol, mannose, salicin, sorbitol and glycerol. Laminarin, fucose, and inulin were not utilised. Differences were noted amongst the isolates with regard to fermentation of sugars and ability to split arbutin both of which are variable characteristics of the type species. Differences were also noted in the range of temperature permitting growth. Using these differences the isolates have been placed in nine Groups as indicated below and the numbers of the isolates comprising these Groups together with their respective cell measurements are also noted.

Key:-

Group No.	No. of Strains	Sugar Fermentation	Arbutin Splitting	Range of Temperature permitting Growth (in °C)
1	2	Glucose, sucrose(wk)	+	4 - 30
2	2	Glucose	+	4 - 42
3	16	Glucose(wk)	+	4 - 37
4	12	Glucose(wk)	+	4 - 30
5	2	None	+	4 - 42
6	22	None	+	4 - 37
7	32	None	+	4 - 30
8	5	None	-	4 - 37
9	4	None	-	4 - 30

Grouping of isolates and their respective cell measurements in μ :-

Group No.	Strain No.	on malt wort agar			in malt wort		
		lower limits	mean	upper limits	lower limits	mean	upper limits
1	132	2.5,3.0	3.1 x 3.7	5.0,5.5	3.0,3.5	3.8 x 4.4	5.0,5.5
	176	2.5,3.0	3.1 x 3.7	4.0,4.5	2.5,3.0	3.8 x 4.3	5.0,6.0
2	347	2.0,2.0	3.2 x 3.7	4.5,5.0	2.5,2.5	3.2 x 3.7	5.0,5.5
	360	2.5,2.5	3.6 x 4.0	5.5,5.5	2.5,2.5	3.3 x 3.7	5.0,5.0
3	7	3.0,3.5	3.5 x 4.0	4.0,5.0	2.5,3.5	3.2 x 4.1	4.5,5.0
	11	2.0,2.0	2.9 x 3.2	4.0,5.0	1.5,1.5	2.8 x 3.2	4.0,6.5
	15	3.0,3.5	3.7 x 4.8	4.5,6.0	2.5,3.5	3.6 x 4.3	4.5,5.5
	43	3.0,3.5	3.6 x 4.0	5.0,5.5	2.5,4.0	3.8 x 4.2	5.0,5.5
	46	3.0,3.5	3.8 x 4.3	5.5,6.0	2.5,3.5	4.0 x 4.6	5.0,5.5
	49	2.0,2.5	3.9 x 4.2	5.5,6.0	2.5,2.5	3.8 x 4.3	5.5,5.5
	53	2.5,2.5	3.3 x 3.6	4.5,6.0	1.5,2.0	3.2 x 3.3	4.5,5.5
	130	2.5,2.5	3.6 x 3.9	5.0,5.0	2.0,2.5	3.8 x 4.4	5.0,6.0
	131	2.5,2.5	3.6 x 3.4	6.0,6.5	2.5,2.5	3.2 x 3.7	5.5,5.5
	146	2.5,2.5	3.8 x 4.1	5.0,5.5	2.5,3.0	4.0 x 4.4	5.5,7.0
	302	2.0,2.0	3.9 x 4.3	5.0,5.0	2.5,2.5	3.5 x 3.7	5.0,5.0
	319	3.0,3.0	3.1 x 3.8	4.5,5.0	3.0,3.0	3.4 x 3.8	4.0,5.0
	341s	2.0,2.0	3.4 x 3.7	4.5,5.0	2.0,2.5	3.3 x 3.7	4.5,4.5
	371	1.5,3.0	3.8 x 4.4	5.5,6.0	2.5,3.0	3.6 x 4.1	5.0,5.5
	212	3.5,3.5	3.8 x 4.3	5.0,5.5	2.5,3.0	3.7 x 4.9	5.0,6.0
	235	2.0,2.0	3.3 x 3.5	5.5,5.5	2.5,3.0	3.4 x 3.9	4.5,5.5
4	164	2.5,2.5	2.9 x 3.2	4.5,5.0	2.5,2.5	3.4 x 3.7	5.5,5.5
	169	2.5,2.5	3.5 x 3.9	5.0,5.5	3.0,3.0	3.8 x 4.2	5.0,7.0
	183	1.5,2.0	3.4 x 3.7	4.5,5.0	2.5,2.5	3.7 x 4.0	5.5,6.0
	185	2.5,2.5	3.7 x 4.1	5.5,6.5	2.5,2.5	3.8 x 4.1	5.5,6.0
	195	2.5,3.5	3.5 x 3.9	4.5,5.0	3.0,3.0	3.7 x 4.3	5.0,6.0
	227	2.0,2.0	3.4 x 4.8	5.0,5.0	1.5,1.5	3.4 x 3.9	5.0,5.5
	338	2.5,2.5	3.5 x 3.8	4.5,5.0	2.0,3.0	3.3 x 3.7	4.0,5.0
	333	2.0,2.0	3.1 x 3.4	5.5,6.0	2.0,2.0	3.1 x 3.6	4.5,5.0
	329w	2.5,3.0	3.4 x 3.9	5.5,6.0	2.5,3.0	3.6 x 3.9	5.0,6.0
	311	2.5,2.5	3.6 x 3.8	5.5,5.5	2.5,2.5	3.5 x 3.7	4.5,4.5
	450w	2.5,3.0	3.6 x 3.9	5.0,5.0	2.5,2.5	3.6 x 3.9	5.0,5.0
	420m	2.5,2.5	3.5 x 3.9	5.0,6.0	2.5,2.5	3.5 x 3.9	5.0,5.5
5	301	2.5,2.5	3.3 x 3.5	5.0,5.5	2.0,2.0	3.4 x 3.5	4.5,5.0
	336	1.5,2.0	3.5 x 3.8	4.5,5.0	2.0,2.5	3.1 x 3.5	4.0,4.5
6	281	3.0,3.0	3.2 x 3.5	4.5,6.0	1.0,2.0	3.0 x 3.2	5.0,7.0
	431	1.5,1.5	2.9 x 3.1	4.5,6.0	2.0,2.0	3.2 x 3.8	5.5,6.5
	Y11	1.0,1.5	3.2 x 3.4	5.0,6.0	1.0,1.5	2.9 x 3.3	5.0,5.0
	Y54	1.5,1.5	3.1 x 3.2	5.0,5.5	1.5,1.5	3.3 x 3.7	4.5,5.5
	Y3	3.0,3.0	3.1 x 3.3	7.0,8.0	3.0,3.0	3.0 x 3.4	10,10

Group No.	Strain No.	on malt wort agar			in malt wort		
		lower limits	mean	upper limits	lower limits	mean	upper limits
6	147	2.5,3.0	4.0 x 4.6	5.5,6.0	2.5,2.5	3.9 x 4.3	5.5,12.0
cntd.	148	2.0,2.5	3.7 x 4.0	5.0,5.5	2.5,3.0	3.6 x 3.9	5.0,5.5
	157	2.5,2.5	2.9 x 3.3	5.0,6.0	2.0,2.5	4.1 x 4.6	5.0,5.5
	177	2.5,2.5	3.2 x 3.5	4.5,5.5	2.5,2.5	3.7 x 3.9	5.0,5.5
	305	2.5,2.5	3.8 x 4.1	6.0,7.0	2.0,2.0	3.3 x 3.6	4.5,5.0
	342	2.0,2.0	3.3 x 3.6	5.0,5.5	2.0,2.0	3.2 x 3.6	5.0,6.0
	343	2.5,2.5	3.6 x 3.8	5.0,5.5	2.5,2.5	3.6 x 3.8	6.0,7.0
	219	2.0,2.5	2.9 x 3.3	4.0,5.0	2.5,2.5	3.0 x 3.9	4.5,5.5
	354s	2.0,2.5	3.5 x 3.8	5.5,6.0	2.5,2.5	3.3 x 3.8	4.5,5.0
	242	2.5,2.5	3.6 x 4.2	5.5,6.0	2.5,2.5	3.3 x 3.8	4.0,4.5
	208	2.5,2.5	3.7 x 4.1	4.5,5.0	2.5,3.0	3.6 x 4.1	4.5,5.0
	348	2.5,3.0	3.5 x 4.0	4.5,5.5	2.5,3.0	3.6 x 4.0	4.5,5.0
	345	2.5,2.5	3.1 x 3.6	5.0,5.0	2.5,2.5	3.6 x 4.0	5.0,5.5
	344	2.5,3.0	3.3 x 3.7	4.5,5.0	3.0,3.0	3.6 x 4.0	5.0,5.5
	321w	2.0,2.0	3.4 x 3.7	4.0,4.5	2.0,2.0	3.6 x 3.8	4.5,5.5
	443w	2.5,2.5	3.6 x 3.9	5.0,6.0	2.0,2.0	3.4 x 3.8	5.0,5.0
	504	2.5,2.5	3.7 x 4.1	5.0,5.5	2.0,2.0	3.9 x 4.2	5.0,5.5
7	34	2.5,2.5	3.8 x 4.1	5.5,7.0	2.0,2.5	3.7 x 4.0	5.0,5.5
	39	2.5,3.5	4.0 x 4.7	4.5,7.3	2.5,3.5	3.7 x 4.4	5.0,5.5
	41	3.0,3.5	4.0 x 4.7	6.0,7.0	3.0,3.5	3.9 x 4.5	6.0,6.5
	50	2.5,2.5	3.6 x 4.2	5.0,7.0	2.5,2.5	3.8 x 4.2	5.0,6.0
	52	2.5,2.5	3.4 x 3.7	4.0,5.0	2.5,2.5	3.9 x 4.4	5.5,6.0
	59	2.5,2.5	3.6 x 4.1	4.5,5.5	2.5,2.5	3.8 x 4.3	5.5,5.5
	63	2.5,2.5	3.5 x 4.1	5.0,6.0	3.0,3.0	3.8 x 4.2	6.0,7.0
	68	2.5,2.5	4.1 x 4.7	5.0,7.0	2.5,3.5	4.0 x 4.8	5.5,6.5
	159	2.5,2.5	3.7 x 4.1	5.5,6.0	2.5,2.5	3.4 x 3.9	5.0,5.5
	161	2.0,2.0	3.2 x 3.4	4.5,4.5	2.0,2.5	4.0 x 4.3	5.0,5.5
	165	3.0,3.0	3.6 x 3.7	4.0,4.5	2.5,2.5	3.8 x 3.9	5.0,5.5
	306	2.5,2.5	3.8 x 4.0	5.0,5.5	2.5,2.5	3.6 x 3.8	5.0,5.5
	307	2.5,3.0	4.0 x 4.3	5.0,6.5	2.5,3.0	3.8 x 4.1	5.0,5.5
	308m	2.5,3.5	3.7 x 4.2	5.0,5.5	3.0,3.5	3.6 x 4.1	5.0,5.5
	317w	2.5,2.5	3.7 x 3.9	4.5,5.5	2.5,2.5	3.6 x 3.9	4.5,6.0
	322	2.0,2.0	3.3 x 3.5	5.0,5.5	2.0,2.0	3.5 x 3.7	4.5,5.5
	331	2.5,3.0	4.5 x 4.8	5.5,6.5	2.5,3.0	4.4 x 5.0	6.5,7.5
	341m	2.0,2.5	3.0 x 3.4	4.5,5.0	2.5,2.5	3.4 x 3.7	4.0,5.0
	207	2.0,2.0	3.1 x 3.5	4.0,4.5	2.5,2.5	3.7 x 4.0	5.0,6.0
	214	2.5,2.5	3.3 x 3.5	5.5,5.5	2.5,2.5	3.0 x 3.6	4.5,5.0
	222	2.0,2.0	3.4 x 3.7	5.0,5.0	2.0,2.0	3.3 x 3.7	5.0,5.0
	224	2.0,2.0	3.2 x 3.6	4.0,4.5	1.5,1.5	3.5 x 3.9	5.5,6.0
	231	2.0,2.0	3.2 x 3.6	4.5,5.0	2.5,2.5	3.7 x 4.1	5.0,5.0
	236	2.0,2.5	2.9 x 3.3	5.0,5.0	2.0,2.0	3.3 x 3.7	4.0,4.5
	246s	3.0,3.0	3.6 x 4.2	5.0,5.5	2.5,3.5	3.7 x 4.3	5.0,5.0
	248w	2.5,2.5	3.9 x 4.4	3.5,5.5	2.5,3.0	4.1 x 4.6	5.5,6.0
	417m	2.5,2.5	3.9 x 4.4	5.5,6.0	2.5,3.0	3.6 x 4.0	5.0,5.5
	421s	2.5,2.5	3.9 x 4.3	5.0,5.5	2.5,3.0	3.5 x 3.9	5.5,5.5
	423m	2.5,2.5	3.3 x 3.8	5.0,5.5	2.5,2.5	3.5 x 3.9	5.5,6.0
	424s	2.0,2.5	3.6 x 3.6	5.0,5.5	2.5,2.5	3.7 x 4.1	5.5,6.5

Group No.	Strain No.	on malt wort ager			in malt wort		
		lower limits	mean	upper limits	lower limits	mean	upper limits
7	427m	2.5,3.0	3.5 x 3.8	4.5,5.0	3.0,3.0	4.0 x 4.3	5.5,6.0
cntd.	428w	2.0,2.5	3.4 x 3.9	5.0,5.5	2.0,2.5	3.3 x 3.8	4.5,5.0
8	24	2.0,2.0	3.4 x 3.9	4.5,5.5	2.0,2.0	3.3 x 3.8	5.0,5.5
	316	2.5,2.5	3.1 x 3.5	4.0,5.0	2.5,3.0	3.7 x 4.2	4.5,5.0
	1114	2.0,2.5	3.6 x 3.7	5.0,5.5	2.0,2.0	3.2 x 3.5	4.5,5.0
	335	1.5,2.0	2.7 x 3.2	4.5,5.5	2.0,2.0	3.3 x 3.8	5.0,5.5
	209	1.5,2.0	2.6 x 2.7	3.5,3.5	2.0,2.0	3.1 x 3.4	4.5,5.0
9	320	2.5,2.5	3.6 x 3.8	5.5,5.5	2.5,2.5	3.8 x 4.0	5.5,6.0
	425	2.5,2.5	3.6 x 3.9	4.5,5.0	3.0,3.0	3.9 x 4.2	6.0,6.5
	419m	2.5,2.5	3.4 x 3.8	4.5,5.0	2.0,2.5	3.6 x 3.9	5.0,6.0
	418	2.5,2.5	3.8 x 4.2	4.5,5.5	2.5,2.5	3.5 x 3.8	5.5,5.5

Strain 103 was lost before all the tests could be performed but resembl-:ed Groups 3 and 4 most closely by fermenting glucose weakly and splitting arbutin.

B) Debaryomyces subglobosus

Type species - after Lodder and Kreger-van Rij(1952)

The nine isolates given this designation resembled the description of the type species except that four were unable to ferment glucose. All required biotin for growth, were unable to split fat or liquefy gelatin, and utilised the following additional carbon compounds - xylose, mannose, raffinose, L-arabinose, D-mannitol, salicin, sorbitol, and glycerol. Fucose, laminarin, and inulin were not utilised. Differences were noted amongst the isolates with regard to range of temperature permitting growth and these together with the differences in sugar fermentation have been used to place the isolates in five Groups as indicated below. The numbers of the isolates comprising these Groups together with their respective cell dimensions are also noted.

Key:-

Group No.	No. of Strains	Sugar Fermentation	Range of Temperature permitting Growth (in ^{o}C)
1	3	Glucose(weak)	4 - 37
2	2	Glucose(weak)	4 - 30
3	2	None	4 - 37
4	1	None	4 - 30
5	1	None	14 - 30

Grouping of isolates and their repective cell measurements in μ :-

Group No.	Strain No.	on malt wort agar			in malt wort		
		lower limits	mean	upper limits	lower limits	mean	upper limits
1	405	3.0,3.5	3.6 x 4.3	4.5,5.5	2.5,4.0	3.7 x 4.3	5.0,6.0
	325	2.0,2.5	3.6 x 3.9	5.0,5.5	2.0,2.5	3.4 x 3.8	5.0,5.0
	327	2.0,2.5	3.0 x 3.5	4.0,4.5	2.0,3.0	3.3 x 3.8	4.0,4.5
2	202	2.0,2.0	3.5 x 3.2	4.5,5.0	2.5,2.5	3.0 x 3.9	4.5,5.0
	204	3.0,3.5	3.9 x 4.4	5.0,5.5	3.0,4.0	3.8 x 3.9	4.5,5.0
3	304	2.0,2.5	3.2 x 3.4	5.5,5.5	2.0,2.5	3.4 x 3.7	5.0,5.5
	303	2.0,2.0	3.4 x 3.7	4.5,5.0	2.0,2.0	3.4 x 3.7	5.0,5.5
4	422m	2.0,2.5	3.3 x 3.6	5.0,5.0	2.5,3.0	3.5 x 3.9	4.5,5.0
5	337	2.0,2.0	3.2 x 3.6	4.5,5.0	2.0,2.0	2.9 x 3.2	5.0,5.0

C) Torulopsis inconspicua

Type species - after Lodder and Kreger-van Rij(1952)

Two of the four isolates given this designation differed from the type species in being able to split arbutin weakly. Differences amongst the isolates were also noted in their ability to ferment glucose - a variable characteristic of the type species - and to assimilate the additional range of carbon compounds tested i.e. xylose, raffinose, L-arabinose, D-mannitol, mannose, salicin, sorbitol, glycerol, fucose, inulin, and laminarin. Differences were also found in vitamin requirements and range of temperature permitting growth. None of the isolates were able to liquefy gelatin, split fat, or produce starch. Using the different results of the tests mentioned above the isolates have been placed in three Groups as indicated below and the numbers of the isolates comprising these Groups together with their respective cell measurements are also noted.

Key:-

Group No.	Sugar Fermentation	Arbutin Splitting	Additional carbon Compounds utilised	Vitamin Requirements	range of Temperature permitting growth(oC)
1	Glucose(wk)	-	Mannose only	Nicotinic acid, Biotin, Thiamine, Pyridoxine.	14 - 42
2	None	+(wk)	Xylose L-arabinose	Biotin, PABA, Pyridoxine(p) Thiamine	4 - 30
3	None	+(wk)	Xylose, Glycerol, Mannitol	Pyridoxine, Biotin, Thiamine(p)	4 - 37

(p) - partial requirement

Grouping of isolates and their respective cell measurements in μ:-

Group No.	Strain No.	on malt wort agar			in malt wort		
		lower limits	mean	upper limits	lower limits	mean	upper limits
1	104	2.0,2.0	2.7 x 3.3	4.0,5.0	2.0,2.5	2.6 x 3.7	4.0,5.0
	150	2.0,2.0	2.6 x 3.4	4.0,5.0	2.0,2.0	2.8 x 3.7	4.0,5.0
2	66	2.0,3.5	3.0 x 4.6	5.0,6.5	1.5,1.5	2.4 x 3.8	4.0,5.0
3	65	2.5,2.5	3.5 x 4.0	5.0,5.0	3.5,4.0	4.2 x 4.8	5.5,6.5

D) Torulopsis inconspicua (var)

The twenty four isolates given this designation differed from the type species - T.inconspicua - by their ability to assimilate potassium nitrate and also many were able to split arbutin weakly. None were able to split fat, liquefy gelatin or produce starch and of the additional carbon compounds tested - raffinose, fucose, inulin, L-arabinose, D-mannitol, salicin, sorbitol, mannose, glycerol and xylose - only the three last mentioned were assimilated. Differences were noted amongst the isolates with regard to vitamin requirements, fermentation of sugars and range of temperature permitting growth as well as the ability to split arbutin. Using these differences the isolates have been placed in nine Groups as indicated below and the numbers of the isolates comprising these Groups together with their respective cell measurements are also noted.

Key:-

Group No.	No. of Strains	Sugar Fermentation	Arbutin Splitting	Vitamin Requirements	Range of Temperature permitting growth (°C)
1	3	Glucose(wk)	-	B, Pyr	4 - 37
2	1	"	+(wk)	B, Pyr	4 - 37
3	2	"	+(wk)	B, Pyr, Th(p)	4 - 37
4	1	None	-	B, Pyr, Th(p)	4 - 37
5	1	"	-	B, Pyr	4 - 37
6	4	"	+(wk)	B, Pyr	4 - 37
7	2	"	+(wk)	B, Pyr	4 - 30
8	6	"	+(wk)	B, Pyr, Th(p)	4 - 37
9	4	"	+(wk)	B, Pyr(p)	4 - 37

(p) - partial requirement B - Biotin Pyr - Pyridoxine Th - Thiamine

Grouping of isolates and their respective cell measurements in μ:-

Group No.	Strain No.	on malt wort agar			on malt wort		
		lower limits	mean	upper limits	lower limits	mean	upper limits
1	160	2.5,2.5	3.4 x 3.9	5.0,5.5	2.5,3.0	3.6 x 3.9	5.0,5.5
	175	2.0,3.0	3.2 x 4.2	4.5,5.0	2.5,2.5	3.8 x 4.5	6.0,6.5
	196	1.5,2.0	3.0 x 3.3	3.5,4.5	3.0,3.0	4.0 x 4.3	5.5,6.5
2	136	2.5,3.0	3.9 x 4.4	5.0,5.5	2.5,3.0	4.4 x 4.6	5.0,6.0
3	181	2.0,2.5	3.6 x 3.9	5.0,5.0	3.0,3.5	4.6 x 4.9	5.5,5.5
	187	2.5,2.5	3.8 x 4.2	4.5,5.5	2.0,2.0	3.7 x 4.3	5.0,7.0
4	194	2.5,2.5	2.6 x 3.8	4.5,3.5	3.0,3.0	4.1 x 4.4	5.5,6.0
5	166	2.0,2.5	3.1 x 3.6	4.5,5.5	2.5,3.0	4.1 x 4.6	6.0,6.0
6	192	2.0,2.5	2.8 x 3.1	4.0,5.0	3.0,3.0	4.2 x 4.7	5.5,6.0
	193	2.0,2.0	3.1 x 3.3	4.0,4.5	3.0,3.0	4.0 x 4.4	5.5,6.0
	197	2.0,2.0	2.6 x 3.0	5.0,5.0	2.5,2.5	4.0 x 4.4	5.0,6.0
	318	3.0,4.0	3.5 x 4.8	4.5,6.0	4.0,4.0	4.3 x 4.8	5.5,6.5
7	167	3.0,3.5	3.8 x 4.6	5.0,5.5	3.0,3.5	3.7 x 4.4	5.0,5.5
	170	3.5,3.0	3.9 x 4.3	5.5,6.0	2.5,2.5	4.3 x 4.7	5.5,6.0
8	107	2.5,2.5	3.3 x 3.8	4.5,5.0	2.5,2.5	3.2 x 3.3	4.5,5.0
	108	2.0,2.0	3.9 x 4.2	5.5,5.5	1.5,2.0	3.0 x 3.4	4.5,5.0
	112	3.0,3.0	3.3 x 3.9	4.5,5.0	1.5,1.5	4.1 x 4.1	5.0,5.5
	115	2.0,2.0	2.7 x 3.2	4.5,5.0	2.0,2.5	4.2 x 4.6	5.5,6.0
	134	2.5,2.5	3.3 x 3.8	4.5,5.5	3.0,3.0	4.3 x 4.6	5.5,5.5
	190	1.5,2.0	3.3 x 4.2	5.0,5.5	2.0,2.5	3.5 x 3.9	5.0,5.0
9	213	2.5,3.5	3.0 x 4.1	5.0,5.5	3.0,4.0	4.2 x 4.7	5.5,6.0
	215	1.5,1.5	2.9 x 3.9	5.0,5.5	2.0,2.0	3.9 x 4.4	5.5,6.0
	240	2.0,2.0	3.3 x 3.9	5.0,6.0	3.0,3.0	4.2 x 4.7	5.5,6.0
	245	2.0,2.0	2.5 x 3.4	4.5,5.0	2.5,3.0	4.3 x 4.8	5.5,6.5

E) Torulopsis candida

Type species - after Lodder and Kreger-van Rij(1952)

Two of the five isolates given this designation differed from the type species in being able to split arbutin i.e. 340 and 330w. All the isolates were able to assimilate the following additional carbon compounds - xylose, raffinose, L-arabinose, D-mannitol, mannose, salicin, sorbitol, glycerol and laminarin. Fucose and inulin were not assimilated. All were unable to split fat, liquefy gelatin, or produce starch. Differences were noted amongst the isolates with regard to vitamin requirements, sugar fermentation(a variable characteristic of the type species), range of temperature permitting growth as well as ability to split arbutin. Using these differences the isolates have been placed in four Groups as indicated below and the numbers of the isolates comprising these Groups together with their respective cell measurements are also noted.

Key:-

Group No.	No. of Strains	Sugar Fermentation	Vitamin Requirements	Range of Temperature permitting Growth (°C)
1	2	Glucose	Biotin	4 - 37
2	1	None	Thiamine(p)	4 - 30
3	1	None	Biotin, Thiamine	14 - 30
4	1	None	Biotin, Thiamine	4 - 37

(p) - partial requirement

Grouping of isolates and their respective cell measurements in μ:-

Group No.	Strain No.	on malt wort agar			in malt wort		
		lower limits	mean	upper limits	lower limits	mean	upper limits
1	42	2.0,3.0	2.3 x 4.4	4.0,6.0	2.0,4.0	3.3 x 5.1	5.5,7.5
	T40	1.5,1.5	2.4 x 3.9	3.5,5.0	2.0,2.0	3.3 x 4.3	4.5,5.0
2	279	1.5,2.0	2.8 x 4.4	3.9,9.0	1.5,1.5	2.8 x 4.0	4.0,10.0
3	340	1.5,2.5	2.2 x 3.5	3.0,5.0	2.0,3.0	3.1 x 4.1	4.0,5.5
4	330w	2.0,2.5	3.1 x 4.0	4.0,5.5	2.5,2.5	3.4 x 4.1	5.5,5.5

F) Torulopsis famata

Type species - after Lodder and Kreger-van Rij (1952)

The five isolates fitting the description of the type species were unable to liquefy gelatin, split fat or produce starch. All were able to assimilate the following additional carbon compounds - xylose, raffinose, L-arabinose, D-mannitol, mannose, salicin, sorbitol, glycerol and laminarin. Only strain 308s assimilated inulin and fucose. Differences were noted amongst the isolates with regard to fermentation of glucose (variable characteristic of the type species), vitamin requirements and the range of temperature permitting growth. Using these differences the isolates have been placed in three Groups as indicated below and the numbers of the isolates comprising these Groups together with their respective cell measurements were also noted.

Key:-

Group No.	No. of Strains	Sugar Fermentation	Vitamin Requirements	Range of Temperature permitting Growth (°C)
1	1	Glucose(wk)	Biotin, Thiamine (p)	14 - 37
2	2	Glucose(wk)	Biotin	4 - 37
3	2	None	Biotin	4 - 30

(p) - partial requirement

Grouping of isolates and their respective cell measurments in μ:-

Group No.	Strain No.	on malt wort agar			in malt wort		
		lower limits	mean	upper limits	lower limits	mean	upper limits
1	230w	1.5,2.5	2.3 x 3.4	4.0,5.0	2.0,3.0	3.0 x 4.0	4.0,5.5
2	308s	2.0,3.0	2.6 x 4.0	4.0,5.0	1.5,3.0	2.6 x 3.7	3.5,5.5
	67	2.5,3.5	3.6 x 4.2	4.5,5.5	2.5,3.5	3.5 x 4.2	5.0,6.0
3	845	1.5,1.5	2.9 x 3.2	4.5,5.0	3.0,3.0	2.0 x 2.7	7.0,10.0
	846	2.0,2.0	3.7 x 3.9	5.5,5.5	1.5,1.5	3.0 x 3.5	5.0,7.0

G) Torulopsis pseudaria (Zsolt) nov.spec.

Type species

MALT EXTRACT - After 3 days at 25°C the cells are globular, 5 - 8μ in diameter, single or in typical "Torulopsis" groups. After one month a sediment and ring are formed and whitish islets are also present.

MALT AGAR - After 3 days at 25°C similar globular cells as found in malt extract are observed but at the same time there appear also lemon-shaped cells. The cell wall of the globular cells is thicker than that of the lemon-shaped cells. The plasma of the globular cells is yellowish and a large fat globule is obvious in each cell. After one month at 20°C the streak is cream coloured, flat and smooth.

DALMAU PLATE CULTURES - No pseudomycelium formed

SPORULATION - No spore formation on sodium-acetate agar

SUGAR FERMENTATION - None

SUGAR ASSIMILATION - Glucose, sucrose, maltose, lactose, galactose +ve

NITRATE ASSIMILATION - +ve

GROWTH WITH ETHANOL AS SOLE CARBON SOURCE - None

ASSIMILATION OF OTHER CARBON COMPOUNDS - Arabinose, xylose, glycerol, dextrine, sorbitol, dulcitol, acetic acid, lactic acid, citric acid, gluconic acid - all +ve. Starch, inulin, succinic acid, malic acid, fumaric acid, tartaric acid - all -ve.

ESCULIN SPLITTING - weakly +ve

Isolates

(a) Strain 43/6 - This strain resembled the type species in many respects but some differences were apparent. After three days at 25°C on malt wort agar the cells appeared oval to elongated-oval, measuring - (2.5,4.0)(3.0 x 5.5)(4.0,6.0)µ - and after a similar period of incubation in malt wort the cells measured - (2.5,4.0) (3.4 x 5.1)(4.5,8.0)µ. After one month at 17/20°C the streak culture on malt wort agar was greyish-white, smooth and shining. Assimilation of ethanol occurred as well as that of all the additio-:nal carbon compounds - xylose, raffinose, L-arabinose, D-mannitol, salicin, sorbitol, glycerol and laminarin - except mannose, fucose and inulin. No vitamins were required for growth and no splitting of arbutin nor starch production was observed. Slight splitting of fat occurred and gelatin was liquefied after two weeks at 25°C. The range of temperature permitting growth was from 4°C to 30°C with optimal growth at 25°C.

(b) Strain 402 - This strain resembled the type species in many respects but some differences were apparent. After three days at 25°C on malt wort agar the cells appeared to be oval to long-oval in shape, measuring - (1.5,2.0)(3.5 x 4.9)(5.0,7.0)µ - and no ring nor islets were present after one month's growth at 17/20°C. Assimilat-:ion of ethanol occurred as well as of all the additional carbon compounds - raffinose, L-arabinose, D-mannitol, mannose, salicin, sorbitol and laminarin - except xylose, glycerol, fucose and inulin. Growth/

Growth occurred without vitamins and no splitting of arbutin, liquefaction of gelatin nor starch production was observed. The range of temperature permitting growth was from 4°C to 30°C with very slow growth occurring at all temperatures.

H) Candida parapsilosis

Type species - after Lodder and Kreger-van Rij (1952).

The twenty-five isolates resembled the type species in many respects although a few differences were noted, i.e., no giant cells were observed in the pseudomycelia; the streak culture on malt wort agar after one month's growth at 17/20°C was white rather than cream-coloured to yellowish; some isolates were able to split arbutin; some isolates were unable to ferment glucose even weakly. None of the isolates could split fat, liquefy gelatin or assimilate fucose. Differences were apparent in vitamin requirements, range of temperature permitting growth as well as in ability to split arbutin, ferment sugar and assimilate carbon compounds and on the basis of these the isolates have been placed in nineteen Groups as indicated below. The numbers of the isolates comprising these Groups together with their respective cell measurements are also noted.

Key:-

Group No.	No.of Strains	Sugar Fermen-:tation	Arbutin Split-:ting	Vitamin Require-:ments	Additional Carbon Cmpds.not utilised*	Range of Temperature permitting growth(°C)
1	1	Glucose Sucrose Maltose Galactose	-	B	Salicin Raffinose Glycerol Inulin	14 - 42
2	4	Glucose Galactose	-	B	Salicin Inulin	4 - 30
3	1	Glucose Galactose	+	B	Salicin Raffinose Inulin	4 - 30

Group No.	No.of Strains	Sugar Fermntn.	Arbutin Splitt--ing	Vitamin Requirements	Additional carbon cmpds. not utilised *	Range of Temperature permitting growth(°C)
4	1	Glucose Galactose	+	B,Th, Pyr	Raffinose	14 - 42
5	1	Glucose	+	B	None	4 - 37
6	1	Glucose	+	B	Inulin	4 - 37
7	1	Glucose	+	B	Salicin	4 - 37
8	1	Glucose	+	B	Salicin Raffinose	4 - 37
9	1	Glucose	+	B	Inulin Laminarin	14 - 42
10	2	Glucose	+	B	Salicin Raffinose Inulin	4 - 37
11	2	Glucose	+	B	Raffinose Inulin Xylose	4 - 37
12	2	Glucose	+	B	Salicin Raffinose Inulin	4 - 30
13	1	Glucose	+	B(p)	Salicin Raffinose Inulin	4 - 30
14	1	Glucose	+	B(p),Pyr(p) Pan(p)	Salicin Raffinose Inulin	4 - 30
15	1	Glucose	+	B,Th, PABA	Salicin Raffinose Inulin	14 - 42
16	1	None	+	B,Pyr,I, Th(p)	Salicin Raffinose Inulin	4 - 30

17/

Group No.	No.of Strains	Sugar Fermtn.	Arbutin Split-:ting	Vitamin require-:ments	Additional carbon cmpds. not utilised *	Range of Temperature permit-:ting growth (°C)
17	1	None	+(wk)	B	Salicin Raffinose	14 - 37
18	1	None	-	Pyr(p)	None	4 - 30
19	1	None	-	B,Th(p)	Raffinose Glycerol mannose Laminarin	4 - 30

B - Biotin Th - Thiamine I - Inositol Pan - Calcium pantothenate
(p) - Partial requirement Pyr - Pyridoxine

* Fucose was not utilised by any of these yeasts.

Grouping of isolates and their respective cell measurements in μ:-

Group No.	Strain No.	on malt wort agar			in malt wort		
		lower limits	mean	upper limits	lower limits	mean	upper limits
1	117	3.0,3.0	4.5 x 5.3	6.0,7.5	3.0,3.0	4.8 x 5.6	7.0,7.0
2	T2	2.5,2.5	3.7 x 4.1	5.0,6.0	2.5,3.0	4.1 x 4.7	6.5,8.0
	T4	1.5,1.5	3.2 x 3.7	4.5,5.5	1.5,2.5	3.7 x 4.3	5.0,9.0
	T6	2.0,2.0	2.1 x 2.2	4.5,6.5	3.0,4.0	4.1 x 4.9	6.0,6.5
	T7	2.0,2.0	2.4 x 3.2	5.0,5.5	2.5,2.5	3.5 x 4.1	5.5,6.0
3	158	2.5,3.5	3.3 x 4.9	4.5,7.0	2.5,2.5	3.4 x 4.3	4.5,5.5
4	120	2.5,3.5	3.7 x 5.0	4.5,6.0	2.5,3.0	3.7 x 4.7	5.0,6.0
5	423s	2.0,2.5	3.0 x 4.5	4.0,5.5	2.5,2.5	3.1 x 4.3	4.5,7.5
6	309w	2.0,3.0	3.1 x 4.7	4.0,6.0	2.0,2.5	3.5 x 4.8	5.0,6.5
7	419s	2.5,3.0	3.2 x 4.1	4.5,5.5	2.0,3.0	3.3 x 4.4	4.0,5.0
8	421H	2.0,2.5	2.6 x 4.1	4.5,6.0	2.0,2.5	3.0 x 4.4	4.0,6.0

9/

Group No.	Strain No.	on malt wort agar			in malt wort		
		lower limits	mean	upper limits	lower limits	mean	upper limits
9	314	2.0,3.5	2.3 x 4.1	3.0,6.5	2.0,3.5	3.1 x 4.3	4.5,5.5
10	191	1.5,3.0	2.7 x 4.5	3.5,6.0	1.5,3.0	2.4 x 3.9	3.5,5.5
	102	1.5,2.5	2.6 x 4.4	3.5,5.5	2.0,2.5	3.2 x 3.9	5.5,5.5
11	LightI	1.5,2.0	3.2 x 5.1	5.0,8.0	2.0,2.0	4.4 x 6.3	6.0,12.5
	LightII	1.5,1.5	3.0 x 5.4	6.0,7.5	1.5,1.5	3.6 x 5.1	5.5,8.5
12	62	2.5,3.0	3.0 x 4.4	4.5,6.0	2.0,2.0	2.7 x 3.5	4.0,5.5
	31	2.0,4.0	2.9 x 4.6	3.0,6.0	3.0,3.0	3.3 x 4.4	4.5,5.5
13	8	2.0,3.0	2.8 x 4.3	4.0,6.0	2.5,3.0	3.2 x 5.8	4.5,5.5
14	2	2.5,2.5	3.6 x 4.6	6.0,7.5	2.5,3.0	4.0 x 4.6	6.0,7.0
15	21	1.5,2.5	2.7 x 5.0	4.0,5.5	2.0,2.5	3.3 x 4.4	4.0,6.0
16	13	1.5,2.5	2.2 x 4.9	3.0,10.0	2.0,3.5	2.8 x 4.4	4.5,8.5
17	422s	2.0,2.0	2.6 x 3.5	3.5,5.0	2.0,3.0	3.1 x 4.1	4.5,5.0
18	420s	2.0,3.5	3.2 x 4.5	5.0,6.5	1.5,3.0	2.8 x 4.5	5.5,6.5
19	429	2.0,2.5	3.1 x 3.9	5.0,6.5	2.0,3.0	3.2 x 4.0	4.0,5.0

I) Candida lipolytica(var)

Type species - after Lodder and Kreger-van Rij(1952)

The ten isolates given this designation differed from the description of the type species in several respects. They were able to assimilate nitrate; true mycelia were absent; a film-like pellicle was produced after one month's growth in malt wort at 17/20°C; cylindrically shaped cells occurred after three days on malt wort agar at 25°C (except strains Her3 and Her7); good growth was obtained in ethanol medium; whitish-grey streak was produced on malt wort agar after one month's growth at 17/20°C having a dull velvety appearance (Her3 and Her7 had whitish-grey streaks with a smooth appearance).

All isolates required thiamine for growth and the range of temperatures permitting growth was 4°C to 30°C. Only mannose and glycerol of the additional carbon compounds tested i.e. xylose, salicin, raffinose, L-arabinose, D-mannitol, sorbitol, laminarin, fucose and inulin, were assimilated (Her3 and Her7 were also able to assimilate inulin and sorbitol). Only Her3 was able to split arbutin.

The numbers of the isolates comprising this species group together with their respective cell dimensions are indicated below:-

Strain No.	on malt wort agar (in μ)			in malt wort (in μ)		
	lower limits	mean	upper limits	lower limits	mean	upper limits
Her3	2.5,2.5	2.8 x 4.2	4.0,5.5	2.5,2.5	3.0 x 4.2	4.5,6.0
Her7	2.5,2.5	3.2 x 3.9	4.5,5.5	2.5,2.5	3.4 x 3.9	5.5,6.0
230	2.5,3.0	3.2 x 4.7	4.0,6.5	2.5,3.0	3.4 x 5.5	4.5,5.5
233	2.5,2.5	3.3 x 4.8	5.5,8.0	2.5,3.5	3.7 x 5.6	5.0,7.0
228	3.0,4.0	3.3 x 4.9	4.0,6.5	3.0,4.5	3.8 x 5.6	5.0,6.5
238	3.0,4.0	4.0 x 5.3	5.0,8.5	3.0,4.5	4.1 x 6.2	5.5,8.5
226	2.0,3.0	3.4 x 5.0	5.0,7.0	2.5,3.0	3.4 x 5.5	4.5,7.0
246 L	2.0,2.5	3.5 x 5.4	5.5,7.0	2.0,3.0	2.9 x 5.2	3.5,6.5
370	1.5,3.0	3.0 x 4.8	5.0,8.0	2.5,3.0	3.4 x 5.6	5.5,9.0
354L	2.0,3.0	3.2 x 5.2	5.5,8.5	2.0,2.5	3.3 x 5.2	5.0,7.0

J) Candida zeylanoides

Type species - after Lodder and Kreger-van Rij (1952) Isolate 249 resembled the type species in most respects except that after three days' growth in malt wort at 25°C the cells were somewhat shorter, measuring - (2.5,3.0)(3.6 x 4.8)(5.5,6.5)μ . After a corresponding period of growth on malt wort agar the cells measured - (3.0,3.0)(4.2 x 5.3)(5.0,6.0)μ . Good growth in ethanol medium was observed. Biotin was required for growth. Weak splitting of fat occurred but no liquefaction of gelatin. Of the additional carbon compounds tested i.e., xylose, raffinose, L-arabinose, salicin, D-mannitol, mannose, sorbitol, glycerol, laminarin, fucose and inulin, only D-mannitol, mannose, sorbitol and glycerol were assimilated. The range of temperature permitting growth was 4°C to 37°C. Litmus milk was not peptonised and a slight alkaline reaction was observed.

K) Candida species

(a) Strain 417s - This strain resembled the type species of C.parapsilosis (Lodder and Kreger-van Rij,1952) except that it possessed the ability to assimilate potassium nitrate. No giant cells were observed in the pseudomycelia and very few blastospores were formed. The streak culture after one month's growth at 17/20°C on malt wort agar was white, shining and somewhat rugose. No splitting of fat or liquefaction of gelatin occurred and after several days' growth at 25°C in litmus milk an alkaline reaction was produced and no peptonisation occurred. Of the additional range of carbon compounds - xylose, raffinose, L-arabinose, D-mannitol, mannose, salicin, sorbitol, glycerol, laminarin, fucose and inulin - only raffinose, laminarin, fucose and glycerol were not assimilated. After three days' growth on malt wort agar at 25°C the cells measured - (2.0,2.5)(2.8 x 3.7) (4.5,6.5) μ and after a similar period of growth in malt wort their measurements were - (2.0,2.5)(2.7 x 3.6)(4.0,5.5)μ. Biotin was required for growth and the range of temperature permitting growth was 4°C to 30°C.

(b) Strains 419H, 420H, 421m, 424H and 427s - A full description of these strains is given below:-

MALT WORT - After 3 days at 25°C the cells were elongated-oval to cylindrical in shape with single,terminal buds. A small flocculent deposit was formed. After one month at 17/20°C a large flocculent deposit and delicate creeping pellicle were present.

MALT WORT AGAR - After 3 days at 25°C the cells had a similar appearance to those in malt wort. The streak after one month at 17/20°C appeared yellowish-brown, glistening and smooth.

SLIDE CULTURES - The pseudomycelium was well developed, long, thin and much branched with only a few blastospores.

SPORE FORMATION - None observed

SUGAR FERMENTATION - None

SUGAR ASSIMILATION - Glucose and sucrose were assimilated by all the isolates. Only 420H, 421m, 424H and 419H assimilated maltose; 419H also assimilated galactose.

NITRATE ASSIMILATION - All +ve

ETHANOL ASSIMILATION - All the isolates assimilated ethanol

SPLITTING OF ARBUTIN - All -ve

ADDITIONAL TESTS - None of the isolates required vitamins for growth and the range of temperature permitting growth was 4°C to 30°C with optimal growth from 14°C to 25°C. All the isolates were able to split fat and liquefy gelatin. Differences were noted in their ability to assimilate the additional carbon compounds - as listed in (a) - and using these together with the differences in the primary assimilation tests the isolates have been placed in three Groups as indicated below. The numbers of the isolates comprising these Groups together with their respective cell measurements are also noted.

Key:-

Group No.	No. of Strains	Assimilation of five sugars of Lodder and Kreger-van Rij (1952)	Additional carbon compounds not utilised
1	1	Glucose, Sucrose, Maltose, Galactose	L-arabinose, fucose glycerol
2	3	Glucose, Sucrose Maltose	L-arabinose, fucose, glycerol, raffinose, laminarin
3	1	Glucose, Sucrose	L-arabinose, fucose, glycerol, raffinose, laminarin, salicin, mannitol, sorbitol

Grouping of isolates and their respective cell measurements in μ:-

Group No.	Strain No.	on malt wort agar			in malt wort		
		lower limits	mean	upper limits	lower limits	mean	upper limits
1	419H	2.5,5.5	3.5 x 7.9	4.5,10	2.0,4.0	3.2 x 7.1	4.5,10
2	420H	2.0,3.0	3.3 x 5.0	5.0,6.5	2.0,4.0	3.8 x 5.7	5.0,7.5
	421m	1.5,4.0	3.0 x 6.5	4.0,8.5	2.5,4.5	3.5 x 7.1	4.5,9.0
	424H	2.5,5.0	3.4 x 6.7	4.5,7.5	2.0,2.5	3.4 x 6.7	4.5,8.0
3	427s	2.5,5.0	3.6 x 7.4	5.0,8.0	2.0,3.0	3.7 x 6.6	5.5,8.0

L) Rhodotorula glutinis var.rubescens

Type species - after Lodder and Kreger-van Rij(1952)

The fifteen isolates given this classification resembled closely the description of the type species. None were able to liquefy gelatin and only strain 40red split fat. Differences were observed amongst the isolates with regard to their ability to split arbutin(a variable characteristic of the type species) and in vitamin requirements, range of temperature permitting growth and assimilation of additional carbon compounds - xylose, raffinose, L-arabinose, D-mannitol, mannose, fucose, salicin, sorbitol, inulin, glycerol and laminarin. Using these differences the isolates have been placed in twelve Groups as indicated below and the numbers of the isolates comprising these Groups together with their respective cell measurements are also noted.

Key:-

Group No.	No.of Strains	Arbutin Split--ting	Vitamin Require--ments	Additional carbon compounds not assimilated	Range of Temperature permitting growth(°C)
1	1	+	None	Salicin	4 - 30
2	1	+	None	Salicin, xylose L-arabinose	4 - 30
3	1	+	None	Salicin, fucose laminarin	4 - 37
4	1	+	None	L-arabinose, sorbi--tol, laminarin, D-mannitol	4 - 37
5	1	-	None	Fucose, laminarin	4 - 30
6 *	1	Not measu--red	Not measu--red	Not measured	4 - 37

Group No.	No. of Strains	Arbutin Splitting	Vitamin Requirements	Additional carbon compounds not assimilated	Range of Temperature permitting growth(°C)
7	1	+	Th(p)	None	4 - 37
8	1	-	Th(p)	Sorbitol, mannitol	4 - 30
9	1	+	Th(p)	Sorbitol, salicin, glycerol, mannitol, laminarin	4 - 37
10	4	+	Th	Mannitol	4 - 37
11	1	-	Th	Mannitol	4 - 37
12	1	+	Th	Sorbitol, mannitol, laminarin	4 - 37

Th - Thiamine (p) - Partial requirement

* Strain 1301 was lost before all the additional tests could be perfo-:rmed - including those for fat splitting and gelatin liquefaction.

Grouping of the isolates and their respective cell measurements in μ:-

Group No.	Strain No.	on malt wort agar			in malt wort		
		lower limits	mean	upper limits	lower limits	mean	upper limits
1	40red	1.5,2.5	3.3 x 3.8	5.0,8.0	1.5,2.0	3.4 x 3.8	5.0,10
2	254	2.0,2.5	3.4 x 4.6	4.5,7.0	2.0,3.0	2.8 x 3.9	9.0,14
3	428p	2.5,3.0	3.4 x 4.9	5.5,6.5	3.0,3.0	3.7 x 5.3	5.0,6.0
4	1112	1.5,1.5	2.7 x 4.1	4.0,7.0	1.5,1.5	3.0 x 4.3	5.0,10
5	422p	2.5,2.5	4.0 x 4.6	5.0,6.5	2.5,2.5	4.4 x 4.9	5.5,6.0
6	1301	2.0,2.0	3.1 x 3.6	4.5,5.5	2.0,2.0	2.8 x 3.4	4.0,5.5
7	1131	2.5,2.5	3.5 x 4.1	5.0,5.5	1.5,1.5	4.0 x 5.3	5.5,10

8/

Group No.	Strain No.	on malt wort agar			in malt wort		
		lower limits	mean	upper limits	lower limits	mean	upper limits
8	423p	3.0,3.0	3.7 x 5.5	5.5,7.0	2.5,4.0	3.4 x 5.6	5.5,7.0
9	317p	2.0,2.0	2.9 x 3.5	4.0,5.5	2.5,2.5	3.4 x 3.9	5.0,5.5
10	302p	3.0,3.0	3.3 x 3.7	4.5,5.0	2.0,2.5	3.4 x 3.8	4.5,5.5
	248p	2.0,3.0	2.7 x 4.5	4.5,5.5	3.0,3.0	3.7 x 5.8	5.5,8.0
	321p	2.0,2.0	3.0 x 3.3	3.5,4.5	2.0,2.5	3.4 x 3.9	5.0,6.0
	332p	2.5,2.5	2.9 x 3.8	4.0,5.0	2.5,2.5	3.3 x 4.4	4.5,6.0
11	309p	2.5,2.5	4.0 x 5.3	5.5,6.0	2.5,3.0	3.9 x 5.4	5.5,7.0
12	443p	2.0,2.5	3.4 x 5.0	4.5,12.5	2.5,2.5	3.5 x 4.8	5.5,7.5

M) Rhodotorula mucilaginosa

Type species - after Lodder and Kreger-van Rij(1952)

The eight isolates given this designation resembled the type species in most respects but some differences were apparent. A delicate creeping pellicle was produced on malt wort after one month at 17/20°C by strains 1111, 329p, 330p, 1110 and 1109 and strain 1111 was unable to split arbutin. All the isolates assimilated ethanol and were able to grow from 4°C to 37°C. No splitting of fat or liquefaction of gelatin was observed. On the basis of differences amongst the isolates with regard to vitamin requirements and assimilation of the additional carbon compounds - xylose, raffinose, L-arabinose, D-mannitol, mannose, salicin, sorbitol, glycerol, laminarin, fucose and inulin - the isolates have been placed in five Groups as indicated below. The numbers of the isolates comprising these Groups together with their respective cell measurements are also noted.

Key:-

Group No.	No. of Strains	Vitamin Requirements	Additional carbon compounds not assimilated
1	2	None	Sorbitol, D-mannitol
2	1	None	Sorbitol, salicin
3	1	Thiamine(p)	Sorbitol, salicin
4	2	Thiamine(p)	Sorbitol, salicin, fucose, mannitol, glycerol, laminarin
5	2	Thiamine	None

(p) - partial requirement

Grouping of isolates and their respective cell measurements in μ:-

Group No.	Strain No.	on malt wort agar			in malt wort		
		lower limits	mean	upper limits	lower limits	mean	upper limits
1	1110	2.0,2.5	2.9 x 3.7	3.5,6.5	2.0,3.0	3.0 x 4.5	4.5,6.0
	1109	2.0,2.0	2.8 x 3.8	4.0,5.0	2.5,2.5	3.2 x 3.9	4.5,5.5
2	1111	2.0,2.5	3.1 x 4.1	5.5,6.0	2.5,2.5	3.3 x 3.7	4.5,5.0
3	12pink	2.0,2.0	2.4 x 3.3	3.5,6.0	2.0,2.0	2.6 x 3.5	4.0,6.0
4	329p	2.0,2.5	3.2 x 4.0	4.5,5.5	2.0,2.5	3.9 x 4.2	4.5,5.0
	330p	2.5,2.5	2.9 x 3.7	4.0,5.5	2.5,2.5	3.2 x 4.4	5.0,5.5
5	427r	2.5,3.0	3.2 x 3.7	4.5,5.5	3.0,3.0	3.9 x 4.2	5.0,6.5
	427p	2.5,2.5	3.0 x 3.6	4.0,5.0	2.5,3.0	3.0 x 3.9	4.0,5.5

N) Rhodotorula rubra

Type species - after Lodder and Kreger-van Rij(1952)

The three isolates given this designation resembled the type species except that no primitive pseudomycelia were formed and slow growth with galactose as the sole carbon source was observed. No gelatin liquefaction or splitting of fat occurred and all three isolates grew from 4°C to 37°C. Strain 1108 required no vitamins for growth and the other two strains both required thiamine. Strain 1108 also differed from the other two strains in being unable to assimilate D-mannitol, sorbitol and salicin from the range of additional carbon compounds tested - xylose, raffinose, L-arabinose, D-mannitol, salicin, sorbitol, glycerol, laminarin, inulin, mannose and fucose - whereas strains 450p and 424p could not assimilate laminarin, D-mannitol and sorbitol. Differences in cell measurements were also observed. The isolates given this classification together with their respective cell measurements are noted below:-

Strain No.	on malt wort agar (in μ)			in malt wort (in μ)		
	lower limits	mean	upper limits	lower limits	mean	upper limits
1108	2.0,2.5	3.2 x 4.7	4.0,7.5	2.0,2.5	3.6 x 5.3	5.0,8.5
450p	2.5,3.0	3.6 x 5.2	5.0,12.5	2.5,2.5	3.7 x 5.3	5.0,7.0
424p	3.0,3.5	3.7 x 5.3	4.5,7.5	2.5,3.5	4.0 x 5.7	5.5,7.5

c) Rhodotorula minuta

Type species - after Lodder and Kreger-van Rij(1952) Strain 417p given this designation resembled the type species in most features except that somewhat larger cells, oval to cylindrical in shape, were produced after three days' growth in malt wort - (3.0,4.0)(3.9 x 6.0)(5.0,8.0)μ - and on malt wort agar the cells measured - (2.5,3.0)(3.1 x 5.8)(4.0,8.5)μ. After one month's growth at 17/20°C in malt wort no thin ring was observed and after a similar period of growth on malt wort agar the streak appeared pale pink rather than orange to red. No primitive pseudomycelia were observed. Arbutin was split. Only poor growth was exhibited without thiamine i.e. partial requirement, and growth occurred between 4°C and 37°C. No splitting of fat nor liquefaction of gelatin occurred and of the additional carbon compounds tested - xylose, raffinose, L-arabinose, D-mannitol, salicin, sorbitol, mannose, glycerol, laminarin, inulin and fucose - only laminarin and D-mannitol were not assimilated.

F) Pichia membranaefaciens

Type species - after Lodder and Kreger-van Rij(1952) Isolates 188 and 189 given this designation closely resembled the type species. Weak fermentation of glucose occurred, only one spore per ascus was observed and good growth with ethanol as sole carbon source was exhibited. No splitting of fat or liquefaction of gelatin occurred and of the additional carbon compounds tested - xylose, L-arabinose, D-mannitol, mannose, sorbitol, salicin, glycerol, fucose, inulin, laminarin and raffinose - only mannose and glycerol were assimilated. Good growth occurred without vitamins and the range of temperature permitting growth was from 4°C to 42°C. The size of the cells after three days at 25°C on malt wort agar and in malt wort is indicated below:-

Strain No.	on malt wort agar(in μ)			in malt wort (in μ)		
	lower limits	mean	upper limits	lower limits	mean	upper limits
188	2.5,6.0	3.4 x 7.9	5.0,15.0	2.5,6.0	3.7 x 7.6	5.0,11.5
189	3.0,6.0	3.2 x 7.6	5.5,14.0	2.5,5.7	3.5 x 7.7	5.0,13.0

Q) Trichosporon pullulans

Type species - after Lodder and Kreger-van Rij(1952)

Strain 1113 given this designation resembled the type species in every respect including the ability to split fat. After three days at 25°C on malt wort agar the cells measured - (1.0,3.0)(3.9 x 6.9)(6.5,12.5)μ - and in malt wort - (1.5,2.5)(3.9 x 7.7)(6.0,17.0)μ. No vitamins were required for growth and gelatin was not liquefied. The range of temperature permitting growth was from 14°C to 30°C. Only fucose from the additional carbon compounds tested - xylose, raffinose, inulin, L-arabinose, D-mannitol, mannose, sorbitol, salicin, laminarin, fucose, and glycerol - was not assimilated.

R) Trichosporon cutaneum var. multisporum

Type species - after Lodder and Kreger-van Rij(1952)

Strains 502 and 503 given this designation resembled the type species in every respect. Good growth with ethanol was exhibited and arbutin was split. The strains also exhibited the ability to split fat and to liquefy gelatin(after two weeks at 25°C). Thiamine was partially required for growth and the range of temperature permitting growth was from 4°C to 30°C. Of the additional range of carbon compounds tested - xylose, raffinose, L-arabinose, D-mannitol, glycerol, mannose, inulin, glycerol, laminarin and sorbitol - only laminarin, glycerol and D-mannitol were not assimilated.

S) Cryptococcus albidus

Type species - after Lodder and Kreger-van Rij(1952)

Strain 510 given this designation resembled the type species in most respects. After three days at 25°C on malt wort agar the cells measured - (2.0,2.5)(3.5 x 4.4)(4.5,5.5)μ and in malt wort - (2.5,3.0)(3.9 x 4.5)(5.0,6.0)μ. No growth occurred with ethanol as sole carbon source and splitting of arbutin was weak. No fat splitting nor gelatin liquefaction occurred and of the additional carbon compounds tested - sorbitol, L-arabinose, laminarin, inulin, mannose, raffinose, xylose, D-mannitol, salicin and glycerol - only the last four mentioned were not assimilated. Biotin was partially required for growth and the range of temperature permitting growth was from 4°C to 30°C.

T) Cryptococcus laurentii

Type species - after Lodder and Kreger-van Rij(1952)

Strain 511 given this designation resembled the type species in every respect except that after one month's growth at 17/20°C in malt wort no ring was formed but a thin creeping film was present. Growth occurred with ethanol as sole carbon source and arbutin was split. No fat splitting nor gelatin liquefaction was observed and of the additional range of carbon compounds tested (as for C.albidus) only salicin was not assimilated. Growth occurred from 4°C to 25°C with optimal growth at 14°C. Slow growth occurred at 25°C without vitamins. After three days' growth at 25°C on malt wort agar the cells measured (2.0,2.5)(4.5 x 5.3)(6.0,8.0)μ and in malt wort-(3.5,3.5)(4.5 x 5.2)(6.5,8.0)μ.

U) Cryptococcus diffluens

Type species - after Lodder and Kreger-van Rij(1952)

Strains 507 and 407 resembled the type species in some respects but some differences were apparent. After one month's growth at 17/20°C in malt wort only a ring and moderate viscid deposit were formed and 407 also formed a thin creeping pellicle. Assimilation of galactose appeared negative for strain 507 (a weak assimilation of galactose was reported for the type species) and a good positive for strain 407. Strain 407 assimilated ethanol and both strains split arbutin. Neither strain split fat nor liquefied gelatin and the range of temperature permitting growth was from 4°C to 30°C with optimum growth occurring at 14°C. Of the additional range of carbon compounds tested (as for D.kloeckeri) strain 507 could not assimilate raffinose and fucose and strain 407 could not assimilate glycerol, fucose and laminarin.

The cell measurements after three days' growth at 25°C on malt wort agar and in malt wort are reported below:-

Strain No.	on malt wort agar(in μ)			in malt wort(in μ)		
	lower limits	mean	upper limits	lower limits	mean	upper limits
407	3.0,4.0	4.9 x 5.5	6.0,6.5	3.5,4.0	4.9 x 5.7	6.0,7.0
507	2.5,3.5	4.2 x 5.7	6.0,8.0	2.0,3.0	4.5 x 5.2	5.5,7.5

V) Metschnikowia krissii (vanUden and Castelo-branco) nov.comb.

Type species

MORPHOLOGY - After 48 hours at 25°C in isolation broth the cells are round and oval - (4.5 - 5.0) x (6.0 - 11.0)μ and long oval - (4.5 - 6.0) x (11.0 - 13.0)μ, single, in pairs and in small groups. A thin pellicle may form after prolonged incubation. The streak culture on isolation agar after 30 days is yellowish-white, soft, dull or glistening and slightly pointed. A primitive pseudomycelium is formed.

SPORULATION - Asci are formed abundantly on V8 medium. sporulation was not observed in isolation medium, malt agar or corn-meal agar. Asci are club-shaped, 12 - 24μ long, and contain a single needle-shaped ascospore, pointed at both ends and 15 - 21μ long.

FERMENTATION - None

ASSIMILATIONS - Glucose, sucrose, maltose, ethanol, glycerol, salicin and D-mannitol - all+ve. Galactose, lactose, nitrate, raffinose, inulin, D-xylose, L-arabinose, and D-sorbitol - all -ve.

VITAMIN REQUIREMENTS - Biotin and thiamine are necessary for growth

MAXIMUM TEMPERATURE PERMITTING GROWTH - 34°C to 35°C

EXPERIMENTAL PATHOGENICITY - for Daphnia magna

Isolates

The five isolates given this designation resembled the type species in most/

most respects but some differences were apparent i.e. weak fermentation of glucose, requirement of biotin only for growth and no assimilation of ethanol. No fat splitting nor liquefaction of gelatin occurred and growth was observed from 4°C to 37°C. Arbutin was split. Of the additional carbon compounds tested - D-mannitol, mannose, inulin, sorbitol, laminarin, salicin, glycerol, xylose, raffinose and L-arabinose - only the three last-mentioned were not assimilated. In their ability to assimilate inulin and sorbitol the isolates differ from the type species.

The cell measurements after 3 days at 25°C in malt wort and on malt wort agar are indicated below:-

Strain No.	on malt wort agar(in μ)			in malt wort(in μ)		
	lower limits	mean	upper limits	lower limits	mean	upper limits
501	3.0,3.0	4.5 x 6.1	6.0,9.5	4.0,4.0	4.2 x 5.9	6.5,7.5
505	4.0,4.0	4.3 x 5.9	7.5,8.5	3.0,4.0	4.4 x 6.3	7.5,8.5
506	3.0,3.0	4.6 x 6.1	6.5,7.5	3.0,3.5	4.8 x 6.4	6.5,8.0
508	2.5,3.5	4.4 x 5.7	7.5,8.5	3.0,3.0	3.9 x 5.2	5.5,7.5
509	3.0,3.0	4.2 x 5.5	6.5,8.0	4.0,4.5	4.9 x 6.2	6.0,7.5

W) Pullularia pullulans(de Bary)Berkhout(1923),after Wynne & Gott(1956)

Type species

Blastospores 3 -4 x 7 - 11μ; chlamydospores 14μ; arthrospores 3 -4 x 7 - 10μ; cladosporium forms 3 - 5 x 12 - 14μ. Small black colonies with greenish-black surface and aerial mycelia on Sabouraud's, corn-:meal, nutrient and Littman agar. Pigment produced only anaerobically Growth at 37°C. Pellicle in broth with slight sediment. Carbohydra-:tes not fermented.

Isolates

Isolates BY1, BY2 and 117A resembled the type species in producing black mucoid growth on solid nutrient media which later(except 117A) became somewhat woolly in appearance due to formation of hyphae. After one month's growth in malt wort at 17/20°C BY1 and BY2 produced a slight ring and a moderate brown granular deposit and 117A produced a moderate black mucoid deposit. Mycelia and oval, lemon and elonga-:ted yeast-like cells i.e. blastospores,with single polar buds were produced by each isolate. After 3 days at 25°C the blastospores of BY1 and BY2 measured - (2.0,4.0)(3.6 x 4.8)(5.0,7.5)μ and those of 117A - (2.5,4.5)(3.6 x 6.2)(5.0,7.5)μ

No sugar fermentation occurred. Glucose, sucrose, maltose, lactose, galactose and ethanol were assimilated by all three isolates but only BY1 and BY2 assimilated nitrate. No arbutin splitting occurred. Growth occurred from 4°C to 37°C and of the additional carbon compou-:nds tested(as for D.kloeckeri) BY1 and BY2 were unable to utilise raffinose,sorbitol and 117A,raffinose,fucose,laminarin and inulin.

X) Unidentified Strains

(1) Strain 182:-

MALT WORT - After 3 days at 25°C the cells were elongated-oval with monopolar budding and measured - (2.5,2.5)(4.2 x 5.9)(5.5,9.5)μ. After one month at 17/20°C a large flocculent deposit and ring were present.

MALT WORT AGAR - After 3 days at 25°C the cells were round, oval and cylindrical in shape with monopolar budding and measured - (2.5,3.5)(4.3 x 6.6)(6.5,12.5)μ. The streak after one month at 17/20°C appeared white, dull and smooth.

SLIDE CULTURES - The pseudomycelium was well developed, long, thin, with few blastospores.

SPORE FORMATION - Oval or elongated-oval asci were formed on Goradkowa's agar and contained 1 to 4 spores.

FERMENTATION OF SUGAR - Glucose only

SUGAR ASSIMILATION - Glucose, sucrose, maltose, galactose - all +ve
lactose - -ve

ASSIMILATION OF KNO_3 - -ve

ASSIMILATION OF ETHANOL - +ve

SPLITTING OF ARBUTIN - -ve

ADDITIONAL TESTS - Growth occurred from 4°C to 42°C. No fat splitting nor gelatin liquefaction occurred and of the additional carbon compounds tested (as for D.kloeckeri) only raffinose and mannose were assimilated. Biotin, inositol and calcium pantothenate were required as growth factors, the last two only partially.

(2) Strain 179:-

MALT WORT - After 3 days at 25°C the cells were round and oval with monopolar or bipolar buds and measured - (3.0,3.0)(5 x 5.5)(6.5,7.5)μ. After one month at 17/20°C a large floccu-:lent deposit was present.

MALT WORT AGAR - After 3 days at 25°C the cells were similar in appea-:rance to those in malt wort and measured - (2.5,3.5)(5.0 x 5.5)(5.0,7.0)μ. The streak after one month at 17/20°C appeared white, glistening and smooth.

SLIDE CULTURES - No pseudomycelium was formed.

SPORE FORMATION - Round asci were formed on Goradkowa's agar contain-:ing 1 to 4 thick-walled spores with central oil-:droplets.

FERMENTATION OF SUGAR - None

SUGAR ASSIMILATION - Glucose, sucrose, maltose, galactose - all +ve
lactose - -ve

ASSIMILATION OF KNO_3 - +ve

ASSIMILATION OF ETHANOL - +ve

SPLITTING OF ARBUTIN - +ve

ADDITIONAL TESTS - Growth was exhibited from 4°C to 37°C. No split-:ting of fat nor liquefaction of gelatin occurred. Of the addi-:tional carbon compounds tested (as for D.kloeckeri)only xylose, mannose and glycerol were assimilated. Biotin, pyridoxine and thiamine were required for growth, the last mentioned one only partially.